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Composition of Fecal Fat in the Absence of Pancreatic Juice in the Rat.

By

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In the absence of the external secretion of the pancreas, the principal lipolytic enzyme remaining in the digestive tract is in the succus entericus. However, the possibility that lipases may be present in the buccal and gastric secretions and may be produced by bacteria in the rumen of the rat should not be forgotten. Histochemical studies indicate that the intestinal lipase or esterase attacks esters of saturated fatty acids preferentially (GOMORI 1949). The specificity and significance of gastric lipase is moot (WILLSTÄTTER and MEMMEN 1924, GOMORI, DEUEL), buccal lipase has not been thoroughly investigated (DEUEL 1955) and studies have not been made on digestion in the rumen of the rat.

In the present study information was sought regarding the over-all efficiency of the gastrointestinal lipases in the hydrolysis of different types of triglycerides in the absence of pancreatic juice. It was thought that such a study would reveal a difference in the handling of saturated and unsaturated fats in case intestinal lipase is the main lipolytic enzyme remaining in the alimentary tract. It was hoped that the study would also reveal some specific function of pancreatic juice in the hydrolysis of fat which could be used as a test of the lack of pancreatic secretion.

This approach has involved the determination of the unhydrolyzed residue, free fatty acid and soap in the feces when saturated and unsaturated, low melting and high melting, cis and trans isomers of fat and fatty acids were fed to rats with and without exclusion of the pancreatic juice.

Methods.

Twenty-four healthy male rats of the Holtzmann-Rolfsmeyer strain, weighing from 200 to 250 grams, were used. In twelve (PDL), the pancreatic juice was excluded from the intestine by the technique of CLOWES and MACPHERSON (1951). In twelve (SOC), a sham operation was performed as previously described (KARVINEN, LIN and IVY, 1956) in such a way that the bile entered the jejunum at the same level in both groups of rats. Only rats in fair to good general condition were used, the criteria for the selection being published previously.

Each rat was caged individually and fed an equal number of calories. The quantity of the ration was such that it was completely consumed. Each feeding period lasted four days. Two daily rations were placed in the food cup every 48 hours, and the total feces were collected during the last 48 hours of each period.

Diet A consisted of 5 g of corn syrup powder, 2.875 g of vitamin free casein, 940 mg of dried yeast, 675 mg of WESSON (1932) salt mixture, 70 mg of DL-methionine, 780 mg. of alpha cellulose powder, 50 mg of cholesterol and a half-drop of cod liver oil (35 USP units of vitamin A) per rat per day. Diet B was the same but contained no cholesterol. Into these diets, 1.875 millimols (about 1 gram) per rat per day of the synthetic triglyceride to be tested, or 3 × 1.875 millimols of the fatty acid, or an isocaloric amount of the corn syrup (3.0 g), or 1 g of the natural fats were added. Thus the intake of calories, protein, salt and vitamins was constant. The properties of the fats incorporated in the diet are shown in Table 1.

Table 1.

The fats fed and their characteristics.

Fat fed	Fat intake mg per day	Melting point °C	Approx. number of double bonds per fatty acid	Fatty acid composition
Palmitic acid ..	825	62	0	Hexadecanoic acid
Tripalmitin....	865	58	0	" "
Oleic acid	910	liquid at 20°	1.0	cis-9-octadecenoic acid
Triolein	950	liquid at 20°	1.0	" " " "
Trielaidin	950	48	1.0	Elaidic acid is trans-9-octadecenoic
Beef tallow....	1,000	39	0.45	Oleic 35; palmitic 33; stearic 21; linoleic 3; arachidic 1; other lower fatty acids 6; nonsaponifiable 1 per cent
Corn oil.....	1,000	liquid at 20°	1.4	Linoleic 55; oleic 29; palmitic 10; stearic 3; other lower acids 1 and nonsaponifiable material 2 per cent

The feces were dried in vacuum at 70° C for 5 days, the output of two rats were combined, pulverized and extracted with anhydrous ethyl ether in a Soxhlet apparatus for 48 hours. The ether was evaporated and the residue dissolved in a system of HCl, ethanol and petroleum ether. An aliquot was used for titrating the free fatty acids with isobutanolic KOH, according to the method of VAN DE KAMER, TEN BOKKEL HUINNINK and WEYERS (1949). Then an excess of the same KOH was added in order to saponify the unhydrolyzed fat. The excess of alkali was titrated with HCl, using thymol blue as an indicator, and the amount of unsplit fat was calculated (VAN DE KAMER et al.).

Since Diet A contained considerable amounts of cholesterol, we were intrigued by the possibility of esterified fatty acid being released from cholesteryl esters during saponification and being subsequently titrated as fatty acid supposedly bound as glycerides. However, determination of the actual amounts of fecal cholesterol discussed elsewhere (LIN, KARVINEN and IVY 1955) showed that the capacity of the excreted cholesterol to bind fatty acid as esters — assuming that cholesterol were completely esterified — was 68 to 108 micromols of fatty acid per rat per day in the control group and 78 to 117 micromols in the PDL group. The increment in the maximum capacity of cholesterol to bind fatty acid which resulted from the exclusion of pancreatic juice, ranged from 9 to 32 micromols of fatty acid per rat per day. This quantity constitutes only a fraction of the actually found differences in the excretion of unsplit fat between the control and PDL rats. Even if cholesterol should be entirely free in one group and completely esterified in the other, the artefact produced by the titration of the fatty acid thus bound would account for 17 to 70 per cent only of the observed differences between the control and PDL rats.

The same sample of feces which had been extracted with ether was acidified, and re-extracted with ether, and the amount of fatty acids bound as soap was determined gravimetrically after washing as described in a previous report (KARVINEN et al.).

Results.

The data on the fecal lipids of the SOC animals are summarized in Table 2. Corresponding data on the pancreatic duct ligated animals are shown in Table 3. The amounts of the ingested fats excreted in the feces in the unhydrolyzed form are shown in Tables 4 and 5.

Extent of Hydrolysis of the Fats Fed in the Control Group:

The data in Table 4 show that the SOC rats hydrolyzed the different fats almost completely with the exception of tripalmitin. About 26 per cent[1] of the tripalmitin fed was recovered in the

[1] (Table 4) $834 = \frac{x}{100} \times 3 \times 1.075$; $x = 25.8$ %.

Table 2.

Fecal lipids of sham operated (SOC) rats.

Micromols per rat per day (mean ± standard deviation).

Fat ingested	Fatty acid in unhydrolyzed fat (UF)	Free fatty acid (FA)	Soap (S)
Series A			
No fat	22 ± 6 (UF_B)	61 ± 18	48 ± 6
Oleic acid	87 ± 19	139 ± 35	303 ± 45
Triolein	32 ± 21	73 ± 7	100 ± 22
Trielaidin	31 ± 5	136 ± 13	974 ± 181
Palmitic acid	60 ± 55	341 ± 46	2,428 ± 139
Tripalmitin	856 ± 96	268 ± 35	1,006 ± 158
Beef tallow	40 ± 8	115 ± 12	447 ± 39
Corn oil	5 ± 6	75 ± 12	61 ± 14
Series B			
No fat	16 ± 6 (UF_B)	36 ± 3	59 ± 10
Oleic acid	—	—	274 ± 40
Beef tallow	—	215 ± 110	269 ± 134
Corn oil	35 ± 30	49 ± 7	47 ± 10

Table 3.

Fecal lipids of the pancreatic duct ligated (PDL) rats.

Micromols per rat per day (mean ± standard deviation).

Fat ingested	Fatty acid in unhydrolyzed (UF)	Free fatty acid (FA)	Soap (S)
Series A			
No fat	23 ± 20 (UF_B)	69 ± 17	78 ± 22
Oleic acid	115 ± 43	137 ± 21	414 ± 74
Triolein	204 ± 64	272 ± 215	219 ± 60
Trielaidin	188 ± 36	356 ± 222	1,108 ± 166
Palmitic acid	94 ± 31	361 ± 57	2,202 ± 250
Tripalmitin	1,547 ± 202	280 ± 28	868 ± 353
Beef tallow	176 ± 187	206 ± 79	862 ± 228
Corn oil	238 ± 141	273 ± 77	347 ± 231
Series B			
No fat	44 ± 12 (UF_B)	39 ± 5	114 ± 17
Oleic acid	—	—	380 ± 25
Beef tallow	—	229 ± 66	882 ± 227
Corn oil	123 ± 37	132 ± 49	320 ± 152

unhydrolyzed form in the feces. The difference between tripalmitin and other fats studied is statistically significant. This means

Table 4.

Unhydrolyzed residue of fecal fat in the SOC group.

Diet fat	Unhydrolyzed exogenous fat in the feces[1]	Comparison of tripalmitin with fat in the first column, t-values	Comparison of triolein with fat in the first column, t-values
Triolein	10 ± 8	t = 8.95[3]	
Trielaidin	9 ± 12	t = 8.92[3]	t = 0.15
Tallow, series A	18 ± 18	t = 8.80[3]	t = 0.90
Corn oil, series A[2]	−17 ± 59	t = 8.90[3]	t = 1.01
Corn oil, series B[2]	19 ± 11	t = 8.04[3]	t = 1.47
Tripalmitin	834 ± 226		t = 8.95[3]

[1] Micromols of bound fatty acid per rat per day; mean ± standard deviation calculated as UF—UF_B (from Table 2).
[2] Corn oil, series A vs. series B, t = 1.47.
[3] $P < 0.001$.

Table 5.

Unhydrolyzed residue of fecal fat in the PDL group.

Diet fat	Unhydrolyzed exogenous fat in the feces[1]	t-value of the difference between SOC and PDL group in unhydrolyzed fecal fat	Comparison of tripalmitin with fat in the first column, t-values	Comparison of triolein with fat in the first column, t-values
Triolein	181 ± 148	t = 2.60[2]	t = 7.04[3]	
Trielaidin	165 ± 149	t = 2.33[2]	t = 7.13[3]	t = 0.17
Tallow, series A	153 ± 193	t = 1.71	t = 6.93[3]	t = 0.26
Corn oil, series A[4]	215 ± 168	t = 2.90[2]	t = 6.75[3]	t = 0.34
Corn oil, series B[4]	79 ± 44	t = 2.94[3]	t = 7.25[3]	t = 1.47
Tripalmitin	1,524 ± 443	t = 3.08[2]		t = 7.04[3]

[1] Micromols of bound fatty acid per rat per day; mean ± standard deviation calculated as UF—UF_B (from Table 3).
[2] $P < 0.05$.
[3] $P < 0.001$.
[4] Corn oil, series A vs. series B, t = 1.73.

that the high melting saturated triglyceride was not hydrolyzed very successfully by the combined action of all digestive enzymes.

Extent of Hydrolysis of the Fats Fed in the Duct Ligated Group:

In the absence of pancreatic juice, significantly more exogenous neutral fat was eliminated on all fat regimens than in the control group (Table 5).

Table 6.

Effect of excluding pancreatic juice on the amount of unsplit fat in the feces.

	Increment in the excretion of unsplit exogenous fat produced by the exclusion of pancreatic juice[1]	Comparison of tripalmitin with fat in the first column, t-values	Comparison of triolein with fat in the first column, t-values
Triolein	171 ± 101	t = 5.68[3]	
Trielaidin	156 ± 56	t = 6.76[3]	t = 0.26
Tallow, series A	135 ± 237	t = 4.06[4]	t = 0.31
Corn oil, series A[2]	232 ± 210	t = 4.19[4]	t = 0.57
Corn oil, series B[2]	60 ± 55	t = 8.34[3]	t = 2.03
Tripalmitin	690 ± 135		t = 5.68[3]

[1] Micromols of bound fatty acid per rat per day; mean ± standard deviation calculated as

$$\left(UF^{PDL} - UF_B^{PDL}\right) - \left(UF^{SOC} - UF_B^{SOC}\right)$$

[2] Corn oil, series A vs. series B, t = 1.84.

[3] $P < 0.001$.

[4] $P < 0.01$.

Table 7.

Proportion of unsplit fat in total stool lipid.

Fat Fed	Unsplit residue, per cent of total fecal fat[1]		Change in per cent unsplit fat SOC — PDL	P of change[2] SOC — PDL
	SOC	PDL		
Triolein	15.6	29.4	13.8	0.05
Trielaidin	2.7	11.4	8.7	0.01
Tripalmitin	40.2	57.4	17.2	0.1
Tallow, period A	6.6	14.1	7.5	0.1
Corn oil, period A	35.5	27.7	— 7.8	0.4
Corn oil, period B	26.7	21.4	— 5.3	
Oleic acid, period A	16.4	17.3	0.9	
Palmitic acid	2.1	3.5	1.4	
Basal diet, period A	16.8	13.5	— 3.3	
Basal diet, period B	14.4	22.3	7.9	

[1] Calculated as $100 \times \frac{UF}{UF + FA + S}$ Tables # 2 and # 3

[2] Calculated as unpaired t.

The data in Table 7 show that the exclusion of pancreatic juice does not result in a marked accumulation of the products of the hydrolysis of fat in the feces. This is pointed out because if such were the case and reflected the same condition in the small in-

testine, it would shift the equilibrium toward less hydrolysis of triglycerides.

The new observation in this aspect of our study is that more tripalmitin (47 per cent[1] of the intake) was eliminated in an unhydrolyzed form than in the case of the other fats. This difference between tripalmitin and other fats is highly significant. (Table 6.)

Substrate Specificity of Intestinal Lipases:

The unhydrolyzed fat in the feces in the absence of pancreatic juice provides significant information regarding the substrate specificity of the intestinal lipases. As indicated above in reference to Table 5, it is clear that in the absence of pancreatic juice tripalmitin is affected differently from the other fats; *i. e.*, more of it is unhydrolyzed than in the case of the other fats, there being no significant difference between any two of the other fats. (Note "t"-values in columns headed comparison, Table 5.) This shows that the intestinal lipases (lipases other than pancreatic) are capable of hydrolyzing all of the unsaturated fats, even the solid trielaidin, but were not very capable of attacking the saturated fat tripalmitin. Even at the altered intestinal pH, following ligation of the pancreatic duct (HOERNER 1935), the substrate specificity of an enzyme should be essentially similar to its specificity at the ordinary pH of the intestine. Therefore, it is concluded that intestinal lipase in the rat preferentially hydrolyzes unsaturated fatty acid esters.

Substrate Specificity of Pancreatic Lipase:

When the increments in the unsplit exogenous fecal fat produced by the exclusion of pancreatic juice are calculated (Table 6), it is found that tripalmitin behaved differently from the other fats studied. With tripalmitin, the increment following duct ligation is significantly greater than that of the other fats ("t"-values in columns headed "comparison"). This means that the exclusion of pancreatic juice produced a greater decrease in the hydrolysis of tripalmitin — a saturated triglyceride — than in that of the other fats, which are unsaturated fats, including a fat with a high melting point, trielaidin. Since high melting trielaidin was split as completely as were other unsaturated fats in both experi-

[1] (Table 5) $1{,}524 = \frac{x}{100} \times 3 \times 1.075$; $x = 47$ %.

mental groups, it is believed that the pronounced effect of the pancreatic juice on the hydrolysis of tripalmitin is not due to the melting point of the fat, but rather is due to its saturation. Although it has been shown (BORGSTRÖM 1954, MATTSON and BECK 1956) that pancreatic lipase acts on triglycerides of both saturated and unsaturated fatty acids, our findings show that in the actual hydrolysis of triglycerides in the rat intestine pancreatic juice is more important for the hydrolysis of the saturated triglyceride, tripalmitin, than for the hydrolysis of unsaturated fats.

Table 8.

Excretion of split fat.

Diet Fat	Split fat in excess over endogenous excretion of split fat[1]		t-value difference SOC vs. PDL
	SOC	PDL	
Triolein	64 ± 36	344 ± 248	t = 2.72[2]
Trielaidin	1,001 ± 198	1,317 ± 324	t = 2.03
Tallow, series A	453 ± 56	921 ± 203	t = 5.45[4]
Tallow, series B	389 ± 180	958 ± 283	t = 4.15[3]
Corn oil, series A	27 ± 23	473 ± 272	t = 4.70[3]
Corn oil, series B	1 ± 18	299 ± 190	t = 3.82[3]
Tripalmitin	1,165 ± 193	1,001 ± 385	t = 0.93
Oleic acid, series A + B	333 ± 60	404 ± 57	t = 2.10
Palmitic acid	2,660 ± 180	2,416 ± 286	t = 1.77
Tripalmitin vs. triolein	t = 13.20[4]	t = 3.20[3]	
" vs. Tallow A	t = 7.92[4]	t = 0.36	
" vs. Corn oil A	t = 13.12[4]	t = 2.49[2]	
" vs. Trielaidin	t = 1.32	t = 1.40	
Trielaidin vs. Triolein	t = 10.43[4]	t = 5.32[3]	
" vs. Tallow A	t = 5.97[3]	t = 2.32	
" vs. Corn oil A	t = 10.91[4]	t = 4.46[3]	
Triolein vs. Tallow A	t = 12.98[4]	t = 4.01[3]	
" vs. Corn oil A	t = 1.92	t = 0.78	
Tallow A vs. Tallow B	t = 3.86[3]	t = 0.24	
Corn oil A vs. Corn oil B	t = 1.98	t = 1.17	
Oleic acid vs. palmitic acid	t = 27.4[4]	t = 15.4[4]	

[1] Micromols fatty acid per rat per day; mean ± standard deviation; calculated as $(FA + S) - (FA_B + S_B)$.

[2] $P < 0.05$.

[3] $P < 0.01$.

[4] $P < 0.001$.

Split Fat in the Feces of the Control Rats: The data in Table 8 show that in the control animals significantly more free elaidic and palmitic acid were found in the feces when their triglycerides were fed than of the other fatty acids in the case of the other fats fed. This may indicate that the rate of absorption of elaidic and palmitic acid is poor in comparison with the rate by which their corresponding triglycerides are split by the lipases. On the basis of the ratio of unsplit fat to total saponifiable fecal lipid (Table 7), it appears that trielaidin was split readily but absorbed poorly, because only 2.7 per cent of trielaidin in the feces was unhydrolyzed. Tripalmitin was both slowly split and poorly absorbed. The slow hydrolysis of tripalmitin is shown by finding that 40 per cent of the palmitic acid recovered in the feces was in the form of tripalmitin (Table 7). The calculated absorbability of palmitic acid was only 17 per cent,[1] and because of this poor absorbability palmitic acid appeared in the feces.

Split Fat in the Feces of the PDL Rats: When the pancreatic juice was excluded from the intestine, the amount of fecal split fat increased significantly except with tripalmitin (Table 8). In the case of tripalmitin the amount of split fat in the feces was insignificantly diminished. Despite the increase in the absolute amount of split fat, the percentage of splitting of the fat eliminated was diminished significantly in the case of triolein and trielaidin (Table 7). The percentage was diminished in the case of tripalmitin and tallow, but the change was not statistically significant. In the case of corn oil, the percentage of split fat in the feces was increased but not significantly.

With tripalmitin, the ratio of the unsplit residue to the total fecal fat in the PDL group was higher (57 %) than with any other fat and the increase in the percentage of unsplit fat produced by the exclusion of pancreatic juice was higher (17 %) than with any other fat (Table 7) and the absolute amount of split fat in the feces was diminished by the exclusion of pancreatic juice, instead of being increased as with all other fats (Table 9). These observations confirm the conclusion drawn above in connection with substrate specificity of the lipases, namely that the pancreatic juice is more important for the hydrolysis of tripalmitin than for the hydrolysis of the other triglycerides studied.

[1] (Table 8) Amount fed — recovered =

$$3 \times 1.075 - 2{,}660 = \frac{x}{100} \times 3 \times 1.075;\ x = 17\ \%.$$

Table 9.

Effect of excluding pancreatic juice on the excretion of different fats in a split form.

	Increment in the excretion of split exogenous fat produced by the exclusion of pancreatic juice[1]	Comparison of tripalmitin with fat in the first column, t-values	Comparison of triolein with fat in the first column, t-values
Triolein	280 ± 227	t = 2.39[5]	
Trielaidin	316 ± 384	t = 2.07	t = 0.17
Tallow, series A[2]	468 ± 171	t = 3.38[6]	t = 1.06
Tallow, series B[2]	569 ± 393	t = 3.12[5]	t = 1.42
Corn oil, series A[3]	446 ± 260	t = 3.03[5]	t = 0.74
Corn oil, series B[3]	298 ± 200	t = 2.58[5]	t = 0.12
Tripalmitin	−164 ± 347		t = 2.39[5]
Oleic acid, series A[4]...	71 ± 95		
Palmitic acid[4].....	−244 ± 508		

[1] Micromols of fatty acid per rat per day; mean ± standard deviation: calculated as

$$\left[\left(FA^{PDL} + S^{PDL}\right) - \left(FA_B^{PDL} + S_B^{PDL}\right)\right] - \left[\left(FA^{SOC} + S^{SOC}\right) - \left(FA_B^{SOC} + S_B^{SOC}\right)\right]$$

[2] Tallow series A vs. series B, t = 0.52.
[3] Corn oil, series A vs. series B, t = 1.01.
[4] Oleic acid vs. palmitic acid, t = 1.35.
[5] $P < 0.05$.
[6] $P < 0.01$.

The Excretion of Soap: The amounts of soap excreted are shown in Table 10. It may be noted that the amount of fecal soap closely parallels that of the total split fat (Table 8) and that the changes in the amount of the hydrolyzed fecal fat are reflected in the excretion of soap. Accordingly, the quantities of free fatty acid eliminated remain fairly constant (Tables 2 and 3). It is very probable that soap formation occurred in the intestine, though it may possibly have occurred after defecation. It is interesting to observe that there was more soap in the feces of the duct ligated group (Table 10), which should have a lower intestinal pH and less alkali available for saponification than the controls. Also, the contribution of soap to the total hydrolyzed fat (% of the total hydrolyzed fat in the feces) was not significantly diminished after the exclusion of pancreatic juice (Table 11). Thus, the fatty acid liberated had approximately an equal chance of saponification in the absence as in the presence of pancreatic alkali.

Table 10.

Excretion of soap.

Dietary fat	Exogenous fatty acid excreted in soap[1]		Increment in soap formation in PDL rats (SOC—PDL)	t value of the increment SOC—PDL
	SOC	PDL		
Triolein	52 ± 26	141 ± 53	89	3.23[2]
Trielaidin	926 ± 183	1,030 ± 153	104	1.01
Tripalmitin	958 ± 160	790 ± 375	— 168	0.96
Tallow, series A	399 ± 35	784 ± 230	385	4.23[3]
series B	210 ± 148	768 ± 226	558	4.95[4]
Corn oil, series A	13 ± 14	269 ± 223	256	2.91[2]
series B	— 1 ± 16	206 ± 147	207	3.71[3]
Oleic acid series A	255 ± 45	336 ± 74	81	2.29[2]
series B	215 ± 39	266 ± 26	51	2.66[2]
Palmitic acid	2,380 ± 136	2,124 ± 258	— 256	2.12

[1] Micromols of fatty acid bound in soap per rat per day; mean ± standard deviation; calculated as $S—S_B$.

[2] $P < 0.05$.

[3] $P < 0.01$.

[4] $P < 0.001$.

Table 11.

Soap in per cent of split fat.

Diet Fat	Soap per cent of total split fat in feces[1]		Change in per cent soap formation SOC — PDL	t-value of change in per cent soap excretion
	SOC	PDL		
Triolein	57.8	44.6	— 13.3	1.25
Trielaidin	87.7	75.7	— 12.0	2.42[2]
Tripalmitin	79.0	75.6	— 3.4	1.42
Tallow, series A	79.5	80.7	+ 1.2	0.41
Tallow, series B	55.6	79.4	+ 23.8	4.83[3]
Corn oil, series A	44.9	56.0	+ 11.1	1.10
Corn oil, series B	48.9	70.7	+ 21.8	6.88[3]
Oleic acid, series A	68.6	75.1	+ 6.5	2.01
Palmitic acid	87.7	85.9	— 1.8	2.00

[1] Calculated as $100 \times \frac{S}{S + FA}$.

[2] $P < 0.05$.

[3] $P < 0.001$.

The alkali quite obviously came from the diet, or the secretions of the intestinal and colonic mucosa.

General Discussion.

Our data demonstrate that "intestinal lipase" in the rat preferentially hydrolyzes unsaturated fatty acid esters and that pancreatic juice is more essential for the hydrolysis of saturated fatty acid esters. This is contrary to the histochemical studies of GOMORI (1949), which were conducted under artificial conditions as compared to the conditions of our experiment. Moreover, factors involved in emulsification and absorption were absent in his studies. It should be pointed out that the discrepancy between Gomori's and our findings does not arise from the widely different melting points of tripalmitin and the unsaturated fats, because trielaidin, which is composed of the transisomer of oleic acid and which is solid at body temperature approximates triolein in regard to the completeness of its hydrolysis (Table 5) and in regard to the small extent of the impairment of its hydrolysis following the exclusion of pancreatic juice (Table 6).

The extent of the formation of soap in the pancreatic duct ligated animals shows that the opportunity for the formation of soap is the same as in the controls. This should be anticipated, since it was calculated that the diet used contained more than enough calcium, magnesium and iron to form insoluble soaps to the extent observed. The mechanism involved in the formation and elimination of such relatively large quantities of soap require investigation. Either the fatty acid must be passed into the colon quite rapidly and the insoluble unabsorbable soaps formed there, or the fatty acids are rendered insoluble and unabsorbable in the small intestine, or a combination of both. The soap was relatively insoluble and non-irritating, since our duct ligated animals did not have diarrhea but the usual bulky stool.

A comparison of the relative hydrolysis of triolein and tripalmitin might prove to indicate a deficiency of the secretion of pancreatic juice. However, it would be more laborious than the use of a duodenal tube and tests for pancreatic enzymes or the determination of the absorbability of a labeled fat. In a patient with pancreatic achylia an unsaturated fat of low melting point would be the best fat to feed.

Summary and Conclusions.

Twelve rats with exclusion of pancreatic juice (PDL) from the intestine and 12 sham operated (SOC) were fed 5 different tri-

glyceride fats and 2 fatty acids and their feces were analyzed for unsplit fat, soap and free fatty acid.

In the PDL rats unsaturated fats were recovered mainly in split form, whereas a saturated fat, tripalmitin, was not hydrolyzed extensively. Thus intestinal lipases in the rat preferentially hydrolyze unsaturated triglycerides. Whether this reflects the substrate specificity of true intestinal lipase, is not known.

In the sham operated animals, all fats studied, except corn oil, were hydrolyzed to a greater extent than in the PDL group. This indicates that the pancreatic juice is essential in the hydrolysis of saturated as well as unsaturated fat. The impairment in hydrolysis produced by pancreatic duct ligation was more pronounced in the case of tripalmitin than when other fats were fed. This indicates that the pancreatic juice is even more important for the hydrolysis of saturated than of unsaturated triglycerides.

In all experiments, most of the hydrolyzed fat eliminated was recovered as soap in both control and PDL rats. Thus the opportunity for saponification was not altered by the exclusion of pancreatic alkali.

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Experimenteller Erweis der euklidisch-pythagoreischen Eigenstruktur sowie des Geltens einer quadratischen Metrik in der Gesichtsmannigfaltigkeit.

Von

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Inhalt: Einleitung S. 220. — Verweis auf die Struktur der Gesichtsmannigfaltigkeit. Problemstellung S. 221. — Methode und Ergebnisse der Untersuchung S. 224. — Besprechung des Ergebnisses. Sinnesphysiologischer und philosophischer Kommentar S. 229. — Zusammenfassung S. 235. — Literatur S. 236.

Einleitung. Die Frage der Struktur der Sinnesmannigfaltigkeit ist, nach unserer Auffassung, das Grundproblem der Sinnesphysiologie. Das Sinneswahrnehmbare ist primär im Verhältnis zu allem ihn Beschreibenden, auch im Verhältnis zu den ihn wiedergebenden sog. Reiz- oder Erregungsprozessen, und darum muss das Verständnis des Problems der Sinne von diesem Grund, von dem Sinneswahrgenommenen an sich, ihren Anfang nehmen. Diese Auffassung von der Priorität des Sinneswahrnehmbaren im Verhältnis zu seinen begrifflichen Beschreibungen kann als eine Thematisierung, in betreff der Sinneswahrnehmung, der *Kant*ischen Lehre vom menschlichen Verstand aufgefasst werden. Wie in der Kantischen Lehre die »Anschauung« als Grund des menschlichen Verstandes gesetzt ist, und das Denken mitsamt seinen Begriffen als ein hiervon entsprungener Zusatz aufgefasst wird, so verstehen wir, dass auf dem begrenzteren Gebiete der Lehre von der Sinneswahrnehmung die Wahrnehmung an sich

als thematisierte Anschauung, den Grund ausmacht, von dem die ihr entsprechende Begrifflichkeit des Reizes oder Erregungszustandes entsprungen, deduziert (in biologischer Terminologie: entwickelt; siehe des Späteren) worden ist.

Diese das menschliche Wahrnehmen, Denken und seine Begrifflichkeit in ihre auch genetisch natürliche Ordnung stellende Auffassung ist, unseres Erachtens, in ihrer philosophischen Homogeneität befriedigender als die geläufigen traditionellen Darstellungen der Sinnestätigkeit. Der eine von uns hat diese Behauptung prinzipiell schon früher gemacht (1952, 1953) und sie auch sachlich, auf Grund fremder und eigener Versuchsergebnisse zu stützen versucht (1957). Das Ergebnis der Analyse der Versuche der vorliegenden Arbeit stützt, unseres Erachtens, diese Behauptung ganz besonders.

In der vorliegenden Abhandlung wird die Struktur eines bestimmten Teils, eines »Teilraumes« der Gesichtsmannigfaltigkeit analysiert. Das Ergebnis bestätigt einige früher deduzierte und in axiomatischer Form dargestellte Eigenheiten dieser Mannigfaltigkeit. In der sich als linear und euklidisch-pythagoreisch zeigenden phänomenalen Struktur der Gesichtsmannigfaltigkeit liegt, unseres Erachtens, auch eine, wenigstens in diesem Falle geltende Antwort auf ein ziemlich zentrales Problem der Sinnesphysiologie, nämlich auf das Problem der Summation und der Verstärkung, welches Problem gewöhnlich als eine Reizverstärkung behandelt wird.

Verweis auf die Struktur der Gesichtsmannigfaltigkeit. Problemstellung. Die früher verwendete Darstellungsart der phänomenalen Sinnesmannigfaltigkeit und ihrer begrifflichen Wiedergaben oder Abbilder ist ziemlich umständlich. Wir wollen diese Darlegung (1953) hier nicht wiederholen, müssen jedoch, der Verständlichkeit der Analyse der Versuche wegen, auf einiges in ihr verweisen. Die Sinnesmannigfaltigkeit als solche, als die im »Jetzt« des Augenblicks bestehende Phänomenalität, nannten wir eine *extensionale* Mannigfaltigkeit. Sie besitzt die evident wahrzunehmenden extensionalen Dimensionen der Intensität, der Lokalität (wie der Flächengrösse) und der Qualität sowie die phänomenal augenblickliche Zeitlichkeit. Dieser phänomenalen, nur augenblicklich bestehenden, extensionalen Mannigfaltigkeit entspricht eine »zeitenthobene« begriffliche Mannigfaltigkeit, die wir eine *intensionale* Mannigfaltigkeit genannt haben. Die Strukturen dieser beiden Mannigfaltigkeiten sind nun ganz gleich, die Mannig-

faltigkeiten sind isomorph, da die intensionale, auch adäquat genannte Mannigfaltigkeit aus der extensionalen »durch Zeitenthebung«, d. h. durch Elimination, Ausserachtlassen der Zeitdimension entstanden ist. Man kann sagen, dass die intensionale Mannigfaltigkeit ein isomorphes Begriffs*abbild* von der phänomenalen Sinnesmannigfaltigkeit ist. Eine Analyse der Struktur der phänomenalen Grundmannigfaltigkeit ergibt, dass sie, und somit auch ihr isomorphes Begriffsabbild, eine linear-affine und euklidisch-pythagoreische Struktur besitzt. Was dies bedeutet, soll hier nur angedeutet werden; wir verweisen auf die in axiomatischer Form gegebene frühere Darstellung (1953).

Was in dem hier zu behandelnden Fall der Gesichtsmannigfaltigkeit besonders interessiert ist, dass, wie in allen Sinnesmannigfaltigkeiten, so auch hier die phänomenalen Dimensionen, d. h. diejenigen der Intensität, der Lokalität (der Fläche) und der Farbqualität, von einander »unabhängig« sind, was der Struktur nach einer Orthogonalität der begrifflichen Dimensionen der Abbildmannigfaltigkeit entspricht.

Die *Metrik* der Sinnesmannigfaltigkeit hängt nun in einer bestimmten, eigenartigen Weise sowohl mit ihrer linear-pythagoreischen, als mit ihrer durch die phänomenal-extensionalen Unterschiedsschwellen bedingten diskontinuierlichen Struktur zusammen. Wenn wir die den extensionalen Unterschiedsschwellen der Lokalität ($\Delta_e l$) und der Intensität ($\Delta_e i$) entsprechenden begrifflichen Schwellen ($\Delta_i l$ und $\Delta_i i$; die Indizes geben das Extentionale bzw. das Intensionale an) als Masseinheiten nehmen und eine bestimmte Gesichtsflächengrösse bzw. Gesichtsintensität, gemessen mit diesen natürlichen Masstäben, mit ${}_i l$ bzw. mit ${}_i i$ bezeichnen, kann, gemäss der früheren Deduktion, die Metrik der zweidimensionalen intensitäts-flächenhaften Gesichtsmannigfaltigkeit mittels des Ausdrucks $Q(s) = {}_i s^2 = {}_i l^2 + {}_i i^2$ angegeben werden, wo $Q(s)$ die quadratische Form (${}_i s^2$) der (Vektoren-) Metrik des Gebiets ausdrückt. Das Gelten einer quadratischen Form ist der analytische Ausdruck der pythagoreischen Struktur der Mannigfaltigkeit, die Formel setzt also voraus, dass die Gesichtsmannigfaltigkeit in betreff ihrer Dimensionen der Fläche und der Intensität diese Struktur besitze. In den früheren Arbeiten wurde das Gelten der quadratischen Metrik auf Grund der orthogonalen Struktur der Sinnesmannigfaltigkeiten angenommen; ein direkter empirischer Beweis des Geltens hat aber bisher gefehlt.

Eine empirische Untersuchung der Art der Metrik der zweidimensionalen intensitäts-flächenhaften Gesichtsmannigfaltigkeit kann nun folgendermassen vorgenommen werden. Der begriffsintensionalen Grösse ${}_{i}i$ entspricht das phänomenal-extensionale Erlebnis der Gesichtsintensität ${}_{e}i$. Die Masszahl dieser Grösse ist also die Anzahl der in ihr eingehenden Unterschiedsschwellen. Der lokalen Begriffsgrösse ${}_{i}l$ entspricht wieder die Phänomenalität des Gesichtsfeldes ${}_{e}l$, mit einer in derselben Weise bestimmten Masszahl. Wenn die Gesichtsmannigfaltigkeit eine euklidisch-pythagoreische ist, ist die Begriffsgrösse ${}_{i}s$ ein Vektor von der Form ${}_{i}s = {}_{i}l + {}_{i}i$. Nun ist früher detailliert gezeigt worden, dass einer Begriffsgrösse der Summenform von zwei Summanden verschiedener Dimension, die Phänomenalgrösse der Gleichzeitigkeit der Erlebnisse der beiden Dimensionen entspricht; also ${}_{e}s = {}_{e}l : {}_{e}i$, wenn : das Gleichzeitigkeitszeichen ist. Der *Gleichzeitigkeitsobservation* (Doppelobservation) von einer Gesichtsfläche und deren Intensität entspricht also die isomorphe Begriffsgrösse ${}_{i}s$, die als ein Vektor in der von den orthogonal zueinander stehenden Axen ${}_{i}l$ und ${}_{i}i$ aufgespannten zweidimensionalen Mannigfaltigkeit aufzufassen ist.

Über die Metrik dieses die Gleichzeitigkeits- oder Doppelobservation begrifflich abbildenden Vektors und also auch der Gesichtsmannigfaltigkeit zu der er gehört, besagt das Obige aber noch nichts. Wie gesagt, kann man aber vermuten, dass die Metrik die quadratische ist, wegen der »phänomenalen Orthogonalität«, der phänomenalen »Unabhängigkeit« der Dimensionen der Fläche und der Intensität des Gesichtsfeldes (1953). Wenn die quadratische Metrik gelten würde, hätten wir also ${}_{i}s = \sqrt{{}_{i}l^2 + {}_{i}i^2}$, welche Behauptung empirisch geprüft werden kann.

Es gilt also zu untersuchen ob die »natürliche« Masszahl von ${}_{i}s$, d. h. die *Anzahl* der in betreff der Flächengrösse und der Intensität *gleichzeitig* bestimmten Unterschiedsschwellen, welche in einer Beobachtung der Flächengrösse und der Intensität eines ganz bestimmten Gesichtsfeldes eingehen, gleich ist der aus der Formel $\sqrt{{}_{i}l^2 + {}_{i}i^2}$ berechneten Anzahl der in derselben bestimmten Gesichtsfeldobservation eingehenden Schwellen, welche man in der Weise erhält, dass die Anzahlen der Unterschiedsschwellen der Beobachtung der Flächengrösse (${}_{i}l$) und der Intensität (${}_{i}i$) gesondert, *jede alleine für sich* gezählt werden. Die praktische Ausführung der Versuche gestaltete sich folgendermassen.

Methode und Ergebnisse der Untersuchung. Mit Hilfe von zwei, aus Lichtquellen, Linsensystemen und Irisblenden bestehenden Projektionssystemen (hergestellt von ZEISS) wurden in einem dunklen Raum zwei weisse, kreisrunde Lichtflecke auf einen weissen Schirm mit einem Abstand von 20 cm von einander projiziert. Die Versuchsperson betrachtete die Lichtflecke in einer Entfernung von 5 m. Die Flächengrösse und die Intensität der Flecke konnten mit Hilfe der Blenden mit einer genügenden Genauigkeit auf verschiedene Werte eingestellt werden. Die Intensitätsverteilung in den beiden Flächen war ganz gleichmässig. Alle Versuche wurden bei Reizintensitäten von einigen zehn Lux sowie mit Reizflächen, die grössenordnungsgemäss Gesichtswinkeln von etwa 15 bis 30 Winkelminuten entsprechen ausgeführt.

Nach einer Adaptationszeit von 20 Minuten sollte die Versuchsperson Unterschiedsschwellenbestimmungen *einzeln*, d. h. nur in betreff der Flächengrösse (Δl; die Indizen der Intentionalität und der Extentionalität i und e sind im folgenden ausgelassen) oder nur in betreff der Lichtintensität (Δi) ausführen. Weiter sollte sie Unterschiedsschwellen herstellen, die in betreff dieser beiden Dimensionen *gleichzeitig* schwellenmässig waren ($\Delta (l, i)$). Beim Beginn des Versuchs waren die beiden Flecke (der Haupt- und der Vergleichsreiz) in betreff ihrer Flächengrösse und Intensität gleich; der Vergleichsfleck wurde dann in unterschwelligen, gleichmässigen Schritten allmählich in betreff einer der Grössen, der Fläche oder der Intensität, oder gleichzeitig in betreff ihrer beiden vergrössert, bis die Versuchsperson das Erreichen des ebenmerklichen Unterschiedes angab (Herstellungsmethode).

Es wurden verschiedene Versuchsserien an fünf Versuchspersonen (der Autor R. M. B., seine Frau sowie drei Studenten) ausgeführt. Zeitlich war die Folge der einzelnen Versuche in den verschiedenen Serien die untenstehende (die Indizes geben die Ordnungszahlen der Unterschiedsschwellen an, angefangen von dem mit dem Hauptreiz gleichen Erlebnis).

a) $\Delta i_1\ \Delta l_1\ \Delta i_2\ \Delta l_2 \ldots \Delta i_n\ \Delta l_n$.
b) $\Delta l_1\ \Delta i_1\ \Delta l_2\ \Delta i_2 \ldots \Delta l_n\ \Delta i_n$.
c) $\Delta l_1\ \Delta l_2 \ldots \Delta l_n\ \Delta i_1\ \Delta i_2 \ldots \Delta i_n$.
d) $\Delta i_1\ \Delta i_2 \ldots \Delta i_n\ \Delta l_1\ \Delta l_2 \ldots \Delta l_n$.
e) $\Delta (l, i)_1\ \Delta (l, i)_2 \ldots \Delta (l, i)_n$.

Die Hauptversuchsserien waren solche, in denen bei einer und derselben Versuchsperson entweder eine a-Serie (also eine Folge der Einzelbestimmungen von Flächen- und Intensitätsschwellen) und eine e-Serie (eine Folge der Gleichzeitigkeitsbestimmungen der Flächen- und Intensitätsschwellen) (es wurden insgesamt 22 solche Serien gemacht, mit 25 e-Folgen), oder eine d-Serie und eine e-Serie (2 Serien, mit 6 e-Folgen) ausgeführt wurden. Die Anzahl der durchgelaufenen nacheinanderfolgenden Unterschiedsschwellen war in der Kombination der a- und e-Serien 6 bis 9, in der Kombination der d- und e-Serien 3 bis 5.

Die Ergebnisse geben wir teilweise graphisch, teilweise numerisch, mit aus den graphischen Darstellungen berechneten Werten an.

Die Fig. 1 gibt *unten* ein typisches Beispiel der Versuchsserien, welche gemäss den Verfahren a, b, c und d ausgeführt sind. Die wage- und senkrechten Strecken geben die gesondert erhaltenen, den Unterschiedsschwellen entsprechenden Flächen- und Intensitätszunahmen wieder. Wir sehen, dass die einzelnen Schwellenzuwüchse vom Versuchstypus ziemlich unabhängig sind; besonders deutlich wird dies von dem engen Beieinanderliegen der Endpunkte a_3, b_3, c_3 und d_3 demonstriert; die Punkte liegen in einer Entfernung von einander, die kleiner ist als die Distanz der Unterschiedsschwelle.

Im oberen Teil der Fig. 1 sehen wir Versuche, die eine Versuchsperson teilweise mit der Einzelschwellenmethode a, teilweise mit der Gleichzeitigkeitsmethode *e* ausführte. Die *Einzelschwellen* (die Flächenschwellen Δl und die Intensitätsschwellen Δi) werden von den wage- und senkrechten Strecken $a_1 - a_1'$, $a_2 - a_2', \ldots a_6 - a_6'$ bzw. $a_1' - a_0, a_2' - a_1, \ldots a_6' - a_5$ angegeben. Die *Gleihzeitigkeitsschwellen* $\Delta(l, i)$ werden von den Vektoren $e_1 - e_0$, $e_2 - e_1 \ldots e_8 - e_7$ wiedergegeben. In der Treppenfigur der Einzelschwellen entspricht dem Punkt a_1 also ein Versuchspaar, bei dem erst eine schwellenmässige Flächen- und dann eine Intensitätszunahme vorgenommen wurde. Den Punkten a_2, $a_3, \ldots a_6$ entsprechen die folgenden Paare der Schwellenzunahmen. Um die mit diesen in Einzelschritten gemachten Zunahmen zu vergleichenden Gleichzeitigkeitszunahmen berechnen zu können, sind Kreise durch die Punkte $a_1, a_2, \ldots a_6$ gezeichnet worden, mit dem Mittelpunkt a_0. Die den Einzelschrittpaaren $a_1, a_2, \ldots a_6$ entsprechenden e-Werte sind dann durch Interpolation der Werte der Schnittpunkte der e-Vektoren und dieser Kreise berechnet worden.

Wenn in dieser Weise die den a-Werten entsprechenden mittleren e-Werte aus 22 Versuchsserien berechnet werden, erhalten wir die in der Tabelle 1 angegebenen Zahlen (e_a). (In den Parenthesen sind die Mittelwerte dreier völlig von dem Gewöhnlichen abweichenden Versuchsserien angegeben. Die Abweichung könnte darauf beruhen, dass die Versuchsperson in diesen Serien in Wirklichkeit nicht Gleichzeitigkeitsobservationen, sondern an deren Stelle nur Einzelobservationen, sei es der Flächen — oder der Intensitätszunahme, bewerkstelligt hat. Das Naheliegen der Zahlen 0.7, 1.7, 2.7, 3.1, 5.1 an den, in diesem Falle zu erhaltenden

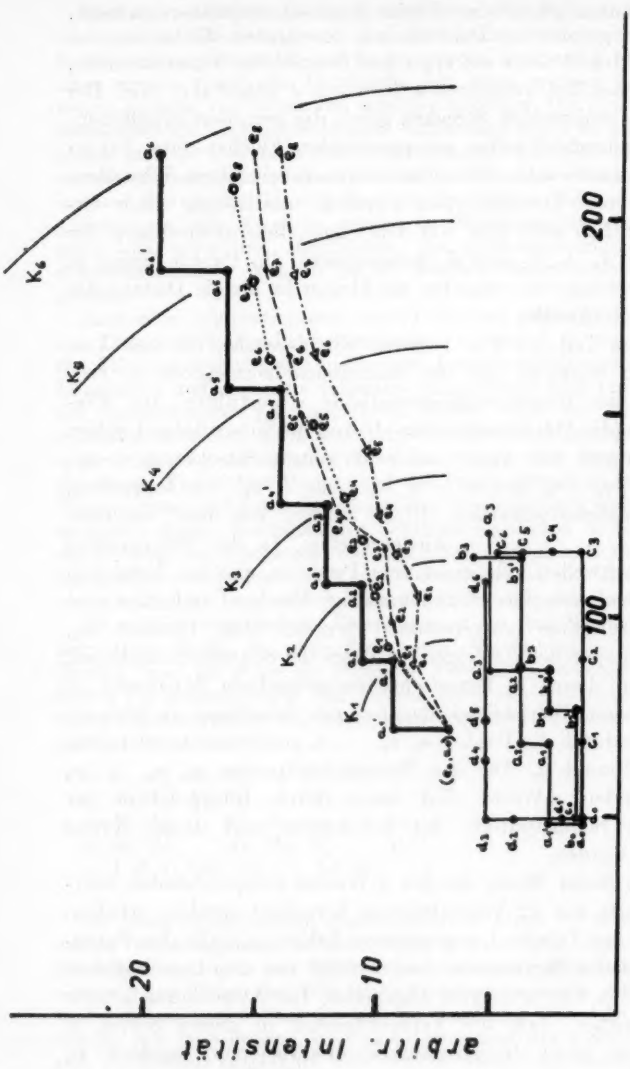

Fig. 1. Graphische Darstellung von Versuchsserien (Vp. R. M. B.), welche teilweise mit der Einzelschwellenmethode (a) der Flächenzunahme (ausgezogene wagerechte Strecken) und der Intensitätszunahme (ausgezogene senkrechte Strecken), teilweise mit der Gleichzeitigkeitaschwellenmethode (e) dieser beiden Dimensionen des Gesichts (gestrichelte und punktierte schräg verlaufende Strecken) ausgeführt sind. Ausführliche Beschreibung im Text.

der Flächenzunahme (ausgezogene wagerechte Strecken) und der Intensitätszunahme (ausgezogene senkrechte Strecken), teilweise mit der Gleichzeitigkeitsschwellenmethode (e) dieser beiden Dimensionen des Gesichts (gestrichelte und punktierte schräg verlaufende Strecken) ausgeführt sind. Ausführliche Beschreibung im Text.

Tabelle 1.

Die Mittelwerte (von 22 Serien) der natürlichen Masszahlen der Gleichzeitigkeitserlebnisse (e_n), die den mit den Paaren der Einzelschwellen erhaltenen Erlebnissen (a_n) entsprechen.

	e_n (Mittelwerte von 22 Serien)	(Mittelwerte von 3 ganz abweichenden Serien)
a_1	1.12 ± 0.59	(0.7)
a_2	2.40 ± 0.50	(1.7)
a_3	3.80 ± 0.34	(2.7)
a_4	5.26 ± 0.39	(3.1)
a_5	7.00 ± 0.39	(5.1)

Zahlen 1, 2, 3, 4, 5 spricht hierfür.) In der Fig. 2 sehen wir ein Beispiel von einer in ganz derselben Weise an einer anderen Versuchsperson ausgeführten Versuchsserie. Die Werte der Versuche der verschiedenen Serien liegen hier sehr nahe an einander.

Ein Vergleich der angegebenen Mittelwerte der e-Serien, welche die proponierten s-Werte, also die Gleichzeitigkeitsvektoren der Metrik sind, mit den aus der pythagoreischen Formel $s = \sqrt{l^2 + i^2}$ berechneten Werten ist in der Tabelle 2 gegeben. Wir sehen, dass die theoretisch aus der Formel berechneten Werte bei den Einzelschwellen 4 und 5 sehr gut mit den experi-

Tabelle 2.

Vergleich der experimentell bestimmten Werte der Masszahlen der Gleichzeitigkeitserlebnisse (Anzahlen der Gleichzeitigkeitsschwellen (s) mit den, mit Hilfe der pythagoreischen Formel aus den Einzelschwellenanzahlen (l und i) erhaltenen theoretischen Werten ($\sqrt{l^2 + i^2}$).

	Versuchsserienanzahl	Anzahl der Einzelschwellen l und i	Entsprechende Anzahlen der Gleichzeitigkeitsschwellen: experimentell bestimmt; s	Entsprechende Anzahlen der Gleichzeitigkeitsschwellen: theoretisch berechnet; $\sqrt{l^2 + i^2}$
a_1	↑	1	1.12	1.41
a_2	22 in allen Serien	2	2.40	2.83
a_3		3	3.80	4.24
a_4		4	5.26	5.66
a_5	↓	5	7.00	7.07

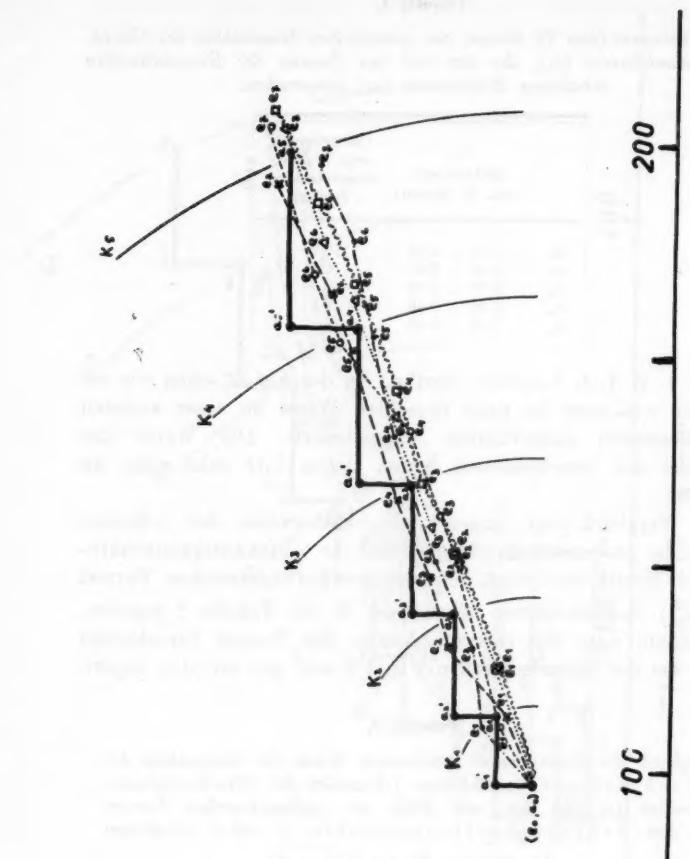

Fig. 2. Versuchsserien der Vp. V. A, ausgeführt in ganz ähnlicher Weise, wie die an der Vp. R. M. B. vorgenommenen, die

Tabelle 3.

Mittelwerte experimentell bestimmter Masszahlen der Gleichzeitigkeitsobservationen ($e_n = s$) verglichen mit aus den Einzelschwellenschritten berechneten theoretischen Masszahlen $\left(\sqrt{l_2 + i^2}\right)$.

	Versuchsserienanzahl	Anzahl der Flächen Intensitäts Schwellen		Entsprechende Anzahlen der Gleichzeitigkeitsschwellen	
		l	i	experimentell bestimmt; s	theoretisch berechnet; $\sqrt{l_2 + i_2}$
d_4	2	3	1	3.0 ± 0.14	3.16
d_5	1	3	2	3.7	3.61
d_6	2	3	3	4.4 ± 0.71	4.24
d_7	1	3	4	4.8	5.00
d_8	6	3	5	5.7 ± 0.14	5.83

mentell bestimmten übereinstimmen, dass die Übereinstimmung aber bei den Werten 1 und 2 weniger gut ist.[1]

Die Fig. 3 gibt schliesslich Versuche von zwei Versuchspersonen wieder, die nach den Methoden d und e ausgeführt sind. Eine Berechnung der e-Werte, die den d-Werten entsprechen, ausgeführt in oben angegebener Weise, sehen wir in der Tabelle 3. Auch hier stimmen die aus der pythagoreischen Formel berechneten Werte gut mit den experimentellen Werten überein.

Besprechung des Ergebnisses. Sinnesphysiologischer und philosophischer Kommentar. Das Ergebnis der empirischen Bestimmung bestätigt also deutlich das Gelten der quadratischen Metrik im untersuchten zweidimensionalen »Raum« des Gesichtssinnes. Der »Gesichtsraum« ist also, ausserdem dass er, auf Grund des Bestehens der Unterschiedsschwellen diskontinuierlich ist, und seine Dimensionen dadurch »das Prinzip ihrer Massbestimmung a priori in sich« tragen, dazu noch von einer euklidisch-pythagoreischen Struktur. Wenn die Massbestimmung in der adäquaten, a prioristischen, natürlichen Weise vorgenommen wird, gilt in diesem Gesichtsraum also die quadratische Metrik. Unsere früher

Fig. 2. Versuchsserien der Vp. V. A. ausgeführt in ganz ähnlicher Weise, wie die an der Vp. R. M. B. vorgenommenen, die Fläche in qmm

[1] Der Grund dieses Verhaltens dürfte der folgende sein. Wenn $i = l\ (= n)$, wie in diesem Falle, ist die Anzahl der Gleichzeitigkeitsschritte $n\sqrt{2}$ und der Unterschied dieser und der Einzelschritte ist $n\sqrt{2} - n$. Eine eventuelle Übereinstimmung der experimentell bestimmten und der theoretisch berechneten Werte (der quadratischen oder der linearen) kann nur in dem Fall hervortreten, dass dieser Unterschied grösser oder mindestens gleich einer Unterschiedsschwelle $(= 1)$ ist. Also nur wenn $n\sqrt{2} - n \geq 1$, d. h. wenn $n \geq 2.5$, also wenn die Anzahl der »Schritte« grösser oder wenigstens gleich 3 ist, ist überhaupt ein Vergleich der experimentellen und der theoretischen Werte möglich.

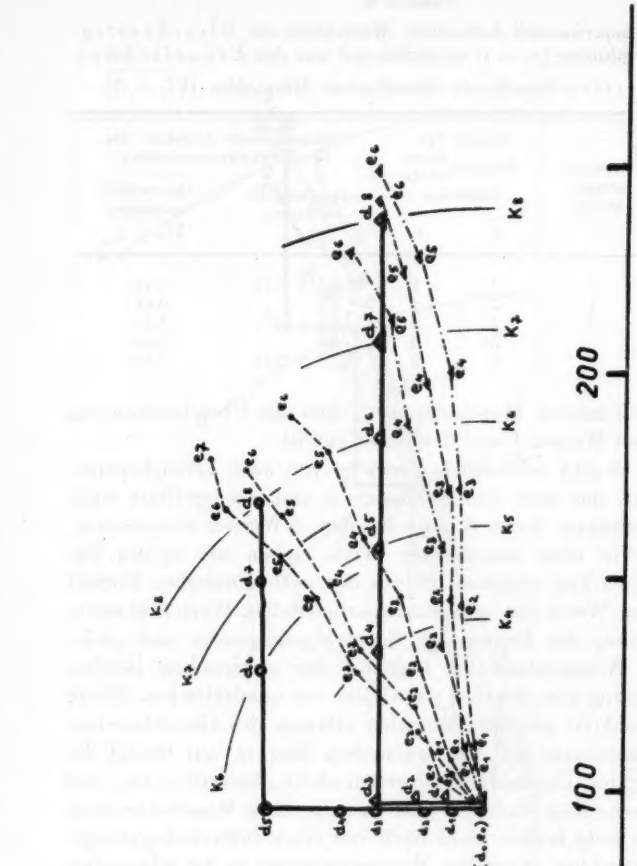

Fig. 3. Graphische Darstellung von Versuchen, die an zwei Versuchspersonen, teilweise gemäss der Einzelschwellenmethode (d) der gesonderten, erst an der Intensität (senkrechte Strecken) nachher an der Fläche (wagerechte Strecken) ausgeführten Unterschiedsschwellenbestimmungen, teilweise gemäss der Gleichzeitigkeitsmethode (e) (gestrichelte schräg verlaufende Strecken) ausgeführt sind.

ausgesprochene Vermutung ist also empirisch bestätigt. Wenn berücksichtigt wird, dass die begriffliche Metrik in sich die Begriffe sowohl der Summe als der Orthogonalität einschliesst, enthält diese Bestätigung auch einen empirischen Beweis der früher aufgestellten Behauptung von der Isomorphie der Strukturen der phänomenalen Gleichzeitigkeit (:) und der begrifflichen Addition (+) sowie der phänomenalen Unabhängigkeit ($_e|$) und der begrifflichen Orthogonalität ($\underline{|}$) (1953).

Das Ergebnis fordert in zweierlei Hinsicht zu Kommentaren auf. Der erste Kommentar betrifft die Darlegung oder Erklärung des erhaltenen Ergebnisses unter Verwendung der »arbiträren« *Reizgrössen* des Flächen- und des Intensitätserlebnisses bei dem Versuch. Wenn wir die arbiträren Reizgrössen mit grossen Buchstaben bezeichnen, kann L die Flächengrösse z. B. in qmm und I die Intensität des Reizlichtes z. B. in Lux bedeuten. Wenn man von dem Erlebnis einer Flächengrösse und einer Intensität, welche einem bestimmten Wertepaar der Reize dieser Grössen L_1, I_1 entspricht, zu dem folgenden, um eine Unterschiedsschwelle sowohl in betreff der Fläche als der Intensität höher gelegenen Erlebnis vorschreitet, mag dem neuen Erlebnis, wenn es gemäss der Methode der gesonderten Einzel-Unterschiedsschwellen der Lokalität und der Intensität bestimmt ist, das Wertepaar L_3, I_3 der arbiträren Reizgrössen entsprechen. Dies ist in der beiliegenden Fig. 4 angegeben. Der in zwei nacheinanderfolgenden Einzelschritten erfolgenden unterschiedsschwellenmässigen, d. h. ebenmerklichen Zunahme der Empfindung entspricht also ein Reizgrössenpaar ΔL, ΔI, wo $\Delta L = L_3 - L_1$ und $\Delta I = I_3 - I_1$ ist. In dem Falle aber, dass wir die Methode des *gleichzeitigen* Erhöhens der Reizgrössen der Fläche und der Intensität verwenden, und entsprechend phänomenal die gleichzeitige Unterschiedsschwelle erleben, sind, wie unser Ergebnis zeigte, die Werte des der höheren Empfindung entsprechenden Reizwertepaares L_2 und I_2 kleiner als im vorigen Falle ($L_2 < L_3$, $I_2 < I_3$; siehe Fig. 4). Die der phänomenalen Gleichzeitigkeitsschwelle entsprechenden arbiträren Reizwerte $\Delta L'$ und $\Delta I'$ sind also kleiner als die Reizwerte der in den einzelnen, nacheinander folgenden Schritten erhaltenen Schwellen; $\Delta L' < \Delta L$ und $\Delta I' < \Delta I$.

Dieses Ergebnis dürfte in der Sprache der herkömmlichen Reizphysiologie wohl in der Weise interpretiert werden, dass das Dargeben der Reize der Fläche und der Intensität (oder der flächenmässigen Excitation der Netzhaut und der Intensität

methode (d) der gesonderten, erst an der Intensität (senkrechte Strecken) nachher an der Fläche (wagerechte Strecken) ausgeführten Unterschiedsschwellenbestimmungen, teilweise gemäss der Gleichzeitigkeitsmethode (e) (gestrichelte schräg verlaufende Strecken) ausgeführt sind.

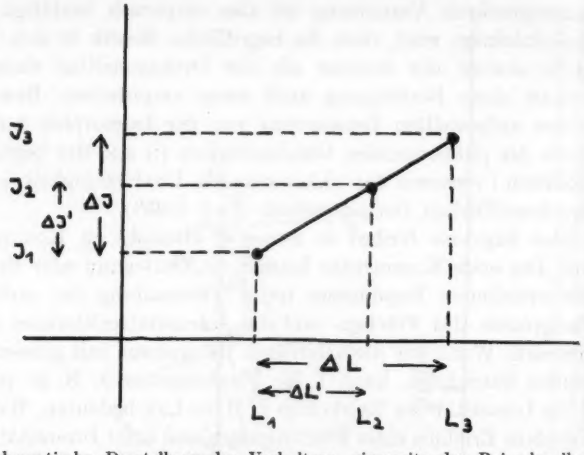

Fig. 4. Schematische Darstellung des Verhaltens, einerseits der Reizschwellen der *gesondert* wirkenden flächenmässigen (Δl) und intensitätsmässigen Excitation (ΔI) bei einer Unterschiedsschwelle des Gesichtssinnes, andererseits der *gleichzeitig* wirkenden Reizunterschiedsschwellen ($\Delta L'$, $\Delta I'$) derselben Dimensionen des Gesichtssinnes.

ihrer Excitation) einander verstärken, eine Summationswirkung haben, so dass ihre gleichzeitige Einwirkung zur Erreichung einer bestimmten Empfindungsgrösse kleinere Werte der Reize beansprucht als ihr gesondertes Dargeben. Das Ergebnis $\Delta L' < \Delta L$ und $\Delta I' < \Delta I$ würde also vielleicht als eine gegenseitige Verstärkung, als eine Summation gleichzeitiger (sonst subliminaler) Reizwirkungen (der in den Rezeptoren stattfindenden Prozesse) verstanden werden.

Unsere Darlegung des Ergebnisses als eines Strukturphänomens der Sinnes-, der Gesichtsmannigfaltigkeit braucht keiner Hypothese der Reiz- oder Kraftwirkungen; sie ist eine direkte Beschreibung der Beobachtungen. Ausserdem erklärt sie das Ergebnis in quantitativer Art, was bei einer Reizverstärkungshypothese, wegen der arbiträren Art ihrer Reize, schwer oder nur in arbiträrer Weise möglich ist. Nur ein von dem »Grund«, des Verhaltens, der phänomenalen Seite des »psycho-physischen« Problems der Sinnesphysiologie, d. h. hier von der Phänomenalität (und nicht von irgendwelchen Reizen oder Erregungen; diese sind, wenn man es biologisch ausdrückt, genetisch späte Begrifflichkeiten im Verhältnis zur frühen Phänomenalität)

ausgehender Erklärungsversuch kann, wie hier gezeigt wurde, eine befriedigende Klärung des Problems geben. Arbiträre Reizhypothesen dagegen, wie die hundertjährige Geschichte der Sinnesphysiologie es wohl zeigt, dürften dies kaum tun können.

Der zweite Kommentar zum Ergebnis betrifft dessen philosophische Bedeutung. Das Gelten einer euklidisch-pythagoreischen Struktur, wie es im Fall des flächenhaft-intensiven Gesichtsfeldes gefunden wurde, kann nicht so verallgemeinert werden, dass wir die Sinnesmannigfaltigkeiten aller Modalgebiete ohne weiteres als pythagoreisch ansehen würden. Die früher gegebene Deduktion der pythagoreischen Natur der begrifflichen Entsprechung (des adäquaten Abbildes) einer beliebigen phänomenalen Mannigfaltigkeit, welche auf der »Unabhängigkeit«, der phänomenalen Orthogonalität der Dimensionen der Mannigfaltigkeit gründet, macht es aber doch sehr wahrscheinlich, dass eine pythagoreische Orthogonalität überall in den Sinnesmannigfaltigkeiten herrscht.

Diese Eigenheit, die »Unabhängigkeit« der Dimensionen der Modalmannigfaltigkeiten, die sich in der pythagoreischen Struktur sowie der quadratischen Metrik ihrer begrifflichen Abbilder wiederspiegelt, bildet ein Problem, dessen Verständnis, unseres Erachtens, nur unter Anleitung der *Kant*ischen Lehre von der Natur des menschlichen Verstandes möglich ist. Man könnte sagen, dass die Kantische Lehre von der Dualität des Verstandes als zeitgebundene Anschauung und zeitenhobene Begrifflichkeit (die gemäss seinen Grundsatz- und Kategorientafeln miteinander konform sind) in diesem Sinnesproblem in einer dem Stoffe des Problems gemässen Thematisierung hervortritt, nämlich in der Dualität der aktual-augenblicklichen Sinnesmannigfaltigkeit und ihrer begrifflichen Abbildstruktur (welche struktural isomorph sind). In dieser formalen Auffassung des Problems liegt aber noch nicht die Lösung der eigentlich philosophischen Seite der Frage. Die formale Analogie ermahnt jedoch in unserem sinnesphysiologischen Fall die philosophische Bedeutung des Verhältnisses zwischen dem Phänomenalen (dem Extensionalen) und dem Begrifflichen (dem Intensionalen) näher zu untersuchen. In Kants Lehre ist das, was er Anschauung nennt, das Basale des menschlichen Verstandes, und der »Begriff« gründet vollkommen in ihr. Das gegenseitige Verhältnis der beiden Bestandteile des Verstandes wird durch die »transzendentale Deduktion« bewerkstelligt; die Begriffe werden aus dem zeitgebundenen Angeschauten (mittels Zeitenthebung) deduziert, ins Zeitlose versetzt.

In unserem konkreten Fall der Sinnesmannigfaltigkeit könnte diese philosophische Darstellung folgendermassen thematisiert werden. Der »Grund«, der »Ursprung« der euklidisch-pythagoreischen Form einer *Begriffs*mannigfaltigkeit liegt in der ihr entsprechenden, isomorphen, zeitlichen, anschauungsartigen (phänomenalen) Mannigfaltigkeit. Die thematisierte Version der Kantischen Anschauung ist in der Sinneslehre also die Sinnesmannigfaltigkeit. Wenn gezeigt wurde, dass diese Phänomenalität, insbesondere die Gesichtsmannigfaltigkeit an sich eine pythagoreische Struktur besitzt, kann in ihr der thematisierte Anschauungsgrund, d. i. der *genetisch* ältere Bestandteil des in exakten Formen arbeitenden Verstandes gesehen werden. Wenn der Mensch bei seiner phylogenetischen Entwicklung seine Sinnesdaten allmählich nicht nur aktual, sondern auch »zeitenthoben« zu verwenden lernt, werden diese neuen, nicht mehr aktual-augenblicklichen, sondern »bestehenden« Verstandesobjekte (die wir Begriffe nennen), da sie direkt aus der Sinneswelt geliehen (enthoben) sind, eine mit dieser Welt isomorphe Struktur erhalten. Die Neoform des Verstandes, welche die Begriffe und das Begriffsdenken umfasst, hat, als die genetisch jüngere, ihre Form aus der älteren Form, der Paläoform, der reinen, ausschliesslichen Sinnesform erhalten.

Diese Auffassung vom »Grund« der euklidisch-pythagoreischen Struktur und der quadratischen Metrik könnte als eine genetische bezeichnet werden. Die Kantische Lehre von der Dualität des Verstandes als Anschauung und Begriff ist auf dem speziellen (exakten) Gebiet der Sinneslehre zu einem Fall der Entwicklungsgeschichte thematisiert worden. Das Gesichtsmannigfaltige, das »ursprünglich« struktural phänomenal-pythagoreisch *Gesehene*, hat sich »später« zu der entsprechend strukturierten begrifflichen Mannigfaltigkeit entwickelt.

Man muss sich fragen, wie sich diese Auffassung zu den von den Mathematikern vorgebrachten Darstellungen der Metrik, speziell der quadratischen Metrik verhält? *R. Nevanlinna* hat in letzter Zeit über das Problem der Metrik der linearen Räume geschrieben. Soweit wir sehen können, besteht kein Widerspruch zwischen seinen rein im Gebiet des Begrifflichen stattfindenden (um die Begriffe der Konformität und der Orthogonalität gebauten) Ausführungen und unserer auch ins Phänomenale sich erstreckenden »genetischen« Darlegung. Wir haben den Eindruck, dass der »Grund« der Metrik einer Begriffsmannigfaltigkeit kaum im

Begrifflichen zu finden ist; wo sollte man ihn denn suchen, wenn nicht im Gebiet des »starken Stammes« unseres Verstandes, im Anschaulich-Phänomenalen; thematisiert: in der Struktur der Sinneswelt? Hier glauben wir ihn denn auch gefunden zu haben.

Die Kantische Lehre vom Gründen des Begrifflichen im Anschaulichen gibt, unseres Erachtens, einen festen Grund zum Verstehen auch ganz spezieller Probleme der einzelnen Beobachtungswissenschaften. Die dargegebene Exposition eines Zentralproblems der Beobachtungswissenschaft par préférence, der Sinnesphysiologie, ist in diesem Sinne eine Thematisierung der dualen Verstandslehre Kants.

Zusammenfassung.

Die von den phänomenalen optischen Dimensionen der Fläche und der Intensität aufgespannte Mannigfaltigkeit ist ein »Teilraum« der Gesichtsmannigfaltigkeit. Es wird experimentell gezeigt, dass die Struktur dieses Gesichtsraumes eine euklidisch-pythagoreische ist. Ihre Eigenmetrik, die durch die Unterschiedsschwellen angegeben ist, ist quadratisch. Die Bedeutung dieses Verhaltens für die Theorie der Sinne wird besprochen, wobei insbesonders gezeigt wird, wie im Falle des »Gesichtsteilraumes« die dargegebene Strukturlehre der Sinne die Reiz-Summationstheorien überflüssig macht. In einem philosophischen Kommentar wird schliesslich versucht, die erwiesene Isomorphie der Strukturen der phänomenalen Gesichtsmannigfaltigkeit und des begrifflichen euklidisch-pythagoreischen Raumes, als eine thematisierte Deduktion (im *Kant*ischen Sinne) der letzteren Mannigfaltigkeit aus der ersteren darzulegen. Eine Verharmlosung der Isomorphie, als eines Beispieles der pythagoreischen Struktur im »Falle« der Gesichtsmannigfaltigkeit möchten wir ablehnen und das Verhältnis von Phänomenalität und Begrifflichkeit, hier der Strukturen des Gesichtssinnes und des euklidisch-pythagoreischen Raumes genetisch, entwickelungsmässig verstehen. Dies dürfte man als eine Thematisierung der Kantischen Lehre von der transzendentalen Deduktion der Begriffe aus der Anschauung, für den speziellen exakten Fall der Sinneslehre, auffassen können.

Zum Schluss danken wir herzlich Herrn Professor ROLF NEVANLINNA für freundliche Hilfe und Kritik bei der Ausformung dieser Abhandlung.

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The Effect of Adrenaline on the Glycogen Metabolism of Smooth Muscle.

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We have shown, in earlier papers, that the stimulating effect of adrenaline on the oxygen consumption (LUNDHOLM 1949), its relaxing effect on smooth muscle (MOHME-LUNDHOLM 1953, 1957) and its vasodilator effect in skeletal muscle (LUNDHOLM 1956) are due to its ability to stimulate the lactic acid production in smooth and striated muscle. Even the stimulating effect of adrenaline on vascular smooth muscle appears to be associated with, and dependent on, breakdown of carbohydrates (LUNDHOLM and MOHME-LUNDHOLM, to be published).

In striated muscle adrenaline causes, concurrently with the increased lactic acid production, a breakdown of glycogen (for review, *vide* CORI 1931). It also has a glycogenolytic effect in the liver (CORI 1931) and heart muscle (CHANG 1936). In the liver, glycogen is broken down into glucose, but in the heart muscle it is converted to lactic acid (GOTTDENKER and MARCHI 1937). It seemed probable, therefore, that in smooth muscle too the stimulating effect of adrenaline on the lactic acid production was associated with a glycogenolytic action. The primary aim of this study was to investigate the effect of adrenaline on the glycogen metabolism of smooth muscle.

In view of the close relationship between the pharmacological effects of adrenaline and its influence on the carbohydrate metabolism, another important aim was to localize the point of interference with the carbohydrate metabolism that led to the increased lactic acid production.

Adrenaline's effect on the glycogen metabolism of smooth muscle was of special interest here, since CORI and SUTHERLAND (1951), in experiments on liver tissue, had found phosphorylase to be the enzyme limiting the velocity of the process: glycogen + phosphate $\overset{\text{phosphorylase}}{\rightleftharpoons}$ glucose-1-phosphate $\overset{\text{phosphoglucomutase}}{\rightleftharpoons}$ glucose-6-phosphate $\overset{\text{glucose-6-phosphatase}}{\rightleftharpoons}$ glucose. In the liver, phosphorylase occurred in an active and an inactive form. Under the influence of adrenaline the content of active phosphorylase rose, so that the glycogen → glucose reaction increased in velocity. This effect was assumed to explain the glycolytic action of adrenaline in the liver as well as its hyperglycemic effect. SUTHERLAND (1951, 1952) later showed that adrenaline also activated phosphorylase in striated muscle. In this type of muscle from rabbit, phosphorylase occurred in two forms: phosphorylase *a*, which was active without addition of adenosine-5-phosphate (AMP), and phosphorylase *b*, which was active only after addition of AMP in a higher concentration than that occurring in skeletal muscle (CORI and GREEN 1943). Under the influence of a factor occurring *e. g.* in muscle extracts — phosphorylase rupturing enzyme (PR enzyme) — phosphorylase *a* → *b* was transformed, and at the same time the molecular weight was halved (CORI and GREEN 1943, CORI and CORI 1945, KELLER and CORI 1953). Under the influence of adrenaline the opposite reaction, phosphorylase *b* → *a*, occurred, but adrenaline did not act by inhibiting the PR enzyme (SUTHERLAND 1951). FISCHER and KREBS (1955) recently succeeded in transforming phosphorylase *b* → *a* *in vitro* too, in the presence of a protein fraction o muscle extract, bivalent metal ions as Ca^{++}, Sr^{++}, Ba^{++} and Mn^{++}, as well as ATP or AMP and phosphocreatine.

If activation of phosphorylase were also responsible for the stimulating effect of adrenaline on the lactic acid production in smooth muscle, then glycogenolysis might be expected to precede the formation of lactic acid, and the muscle's loss of glycogen should be equal to or greater than the amount of lactic acid formed. Substances that inhibited this stimulating effect should inhibit to the same degree the glycogenolytic action of adrenaline too.

With the aim of elucidating these two problems we investigated the effect of adrenaline on glycogenolysis in various types of

smooth muscle — rabbit gut, bovine tracheal muscle and coronary, and guinea-pig uterus — and correlated the results with previously known facts regarding the stimulating effect of adrenaline on the lactic acid production.

The glycogen metabolism in smooth muscle had been very little investigated, probably because the available methods for glycogen assay had not permitted reliable determination of the relatively small amounts present in smooth muscle. A method enabling glycogen contents of down to 25 mg per cent to be determined with satisfactory accuracy was therefore devised at this laboratory (Ek, to be published).

For a better understanding of the effect of adrenaline on the carbohydrate metabolism of smooth muscle, it was also necessary to investigate the velocity of spontaneous glycogenolysis and lactic acid production in various types of unstriped muscle. The results of this investigation will be published later on.

Method.

Rabbit intestine. A rabbit was killed by means of air emboli, and a segment of ileum about 50 cm long was removed and irrigated with Tyrode's solution, then stored in a thermos flask containing iced Tyrode's solution. From this segment were taken two adjacent preparations having a length of 10—15 mm and a weight of 0.6—0.9 g. They were mounted in an organ bath. The activity was registered with an isotonic recorder having a load of 5 g.

Tracheal Muscle from Cow. Ten to fifteen minutes after slaughter, a segment of trachea 20—30 cm long was removed, and the trachealis membranaceus muscle was denuded of cartilage. Only the bands of cartilage at the muscle's insertion were preserved. The preparation was then immersed in a thermos flask containing iced Tyrode's solution. From it were subsequently taken pieces about 10 mm in width and 25—35 mm in length, with a weight of 0.6—1 g, which had been carefully denuded of mucosa and connective tissue. The bands of cartilage at the muscle attachments were retained for the time being, and enabled the preparation to be readily mounted in the organ bath. They were cut away just before the preparations were removed for glycogen assay. The isotonic recorder had a load of 40 g.

Bovine Coronaries. Coronary arteries were dissected free about 20 minutes after slaughter. They were approached and mobilized about 10 mm from the origin in the aorta; then removed with the aid of a sound inserted in the lumen. Tapering segments 5—8 cm in length were obtained, the diameter varying between 4 and 8 mm. The specimens were then immersed in iced Tyrode's solution. The portions immediately adjacent to the aorta were unsuitable for these experi-

ments, since they consisted in great part of elastic tissue. Pieces 10—12 mm in length were taken and opened longitudinally, yielding almost square preparations measuring 10—12 mm on each side and having a weight of 0.1—0.4 g. They were mounted in the organ bath, so that the traction coincided with the direction of the muscle fibers. The isotonic recorder had a load of 2 g.

Guinea-Pig Uterus. Non-pregnant female guinea-pigs weighing about 500 g were killed by a blow on the head. The two uterine horns were dissected free and immediately mounted in separate organ baths. In order that the spontaneous activity would be detectable, the recorder was given as small a load as possible — in these experiments, 2 g. The weight of the uterine horns varied between 0.07 and 0.4 g.

The various organs were mounted in an organ bath *ad modum* MAGNUS. The cups were filled with 20 ml Tyrode's solution having the following composition: 0.8 % NaCl; 0.02 % KCL; 0.02 % $CaCl_2$; 0.02 % $MgCl_2 \cdot 2\,H_2O$; 0.1 % $NaHCO_3$; 0.005 % $NaH_2PO_4 \cdot 2\,H_2O$. Only in cases where it is expressly stated was glucose added. The Tyrode solution was aerated with a mixture of 6.5 per cent CO_2 and 93.5 per cent O_2, and its pH was then 7.1. The experiments were performed at 38° C.

The glycogen content was determined *ad modum* EK. The preparations were immersed in 2 ml of 30 per cent KOH and hydrolyzed for 30 minutes in a boiling water bath. Exactly 0.5 ml of 25 per cent NaCl solution and 9 ml of 99.5 per cent alcohol were then added, after which the mixture was heated to boiling point in a water bath. The precipitate was centrifuged for 10 minutes at 3,500 r. p. m. It was dissolved by heating in 1 ml water, and a further 0.5 ml salt solution was added. This was followed by addition of 9 ml 99.5 per cent alcohol, then heating to boiling point and further centrifugation. The glycogen was precipitated once more in the same way. The final precipitate was dissolved in 5 ml H_2O. Where the total amount of glycogen amounted to 0.5—1.5 mg — *i. e.*, in the experiments with tracheal preparations — 0.2 ml of this solution was taken plus 0.8 ml H_2O and 2.5 ml of a solution containing 4 g diphenylamine, 200 ml glacial acetic acid and 120 ml concentrated hydrochloric acid. The mixture was heated for 45 minutes in a boiling water bath, then cooled; after which the extinction was determined at 6,650 Å and a layer 10 mm thick. Where the total glycogen content amounted to 0.2—0.75 mg, 1 ml of the original glycogen solution plus 2.5 ml diphenylamine reagent was taken instead. Concurrently with determination of the glycogen content of the organs, a standard curve was plotted with a known amount of glycogen. The glycogen content was read from this standard curve.

The method was associated with an error of $\pm$ 5.5 per cent, this figure being uniform at varying glycogen contents. Further, a variation occurred due to a disparity in the glycogen content and the velocity of spontaneous glycogenolysis in the different, contiguous preparations. In four different experiments on tracheal muscle the glycogen content was determined in three contiguous preparations which had been mounted simultaneously in the organ bath for 30 minutes, during the last 15 minutes of which they had been under the influence of carbaminoyl-

choline $2.5 \cdot 10^{-7}$ (5 μg/20 ml). The mean glycogen content was 306 mg per cent (n = 12), and the variation between the different contiguous preparations $\pm$ 26.9 mg per cent, *i. e.*, $\pm$ 8.8 per cent of the mean. The spontaneous variations in the glycogen content were large in relation to the effect of adrenaline on that content. Hence a relatively great number of experiments was necessary for statistical verification of different effects.

Results.

A. *Rabbit Intestine.* The experimental procedure was as follows. Two preparations were mounted in the organ bath. After 10—20 minutes, when they both showed constant activity, adrenaline was added to one of them in a concentration of $1 \cdot 10^{-6}$ (20 μg/20 ml). The tonus fell, and the spontaneous activity ceased within 10 seconds. Subsequently the tonus remained completely inhibited. The change in glycogen content was determined 1 minute and 5 minutes after addition of adrenaline. The experiments were performed both in Tyrode's solution without glucose and in similar solution containing 0.1 per cent glucose. The results are shown in table I. The basal glycogen content averaged 66 mg per cent, the range being 47—87 mg per cent. In preparations immersed in Tyrode's solution containing glucose, the glycogen content scarcely seemed to have changed either 1 minute or 5 minutes after addition of adrenaline. In glucose-free Tyrode's solution, however, a slight though — at all events after 5 minutes — statistically highly probable fall of the glycogen content was observed. This fall amounted after 5 minutes to 4.6 $\pm$ 1.46 mg per cent. Analysis of variance showed a statistically probable difference in the changes of glycogen content between the experiments with and without glucose.

In corresponding experiments on rabbit gut in Tyrode's solution containing 0.1 per cent glucose (MOHME-LUNDHOLM 1953), adrenaline in a concentration of $1 \cdot 10^{-6}$ produced an increase of the lactic acid content amounting, on the average of 12 experiments, to 21.6 $\pm$ 1.6 mg per cent 30 seconds after its addition. After 9 minutes the relevant increase amounted to 13.3 $\pm$ 3.3 mg per cent.

It was accordingly evident that although adrenaline had a glycogenolytic effect in rabbit gut, the glycogen loss was too small to have been responsible for all the lactic acid produced. Nor did the glycogenolysis and lactic acid production coincide; the latter preceded the former. Lastly, the presence of glucose

Table I.

The effect of adrenaline (10^{-6}) on the glycogen content of rabbit intestine. The intestine was analyzed 1 and 5 min. after the addition of adrenaline. P = the probability that the effect was due to chance.

	Basal value mg per cent	Deviation from basal value mg per cent	Number of tests	P
Without glucose:				
after 1 min...	69.7	-2.34 ± 1.23	14	0.1 —0.05
after 5 min...	62.7	-4.64 ± 1.46	14	0.01—0.001
With glucose:				
after 1 min...	68.9	-1.02 ± 1.74	14	0.6 —0.5
after 5 min...	62.9	$+0.39 \pm 1.86$	14	0.9 —0.8

Variance quotient: $\frac{\text{mean square between tests with and without glucose}}{\text{mean square of the error}}$

$= 5.43 \quad 0.05 > P > 0.01$

might totally obscure or prevent the glycogenolytic effect of adrenaline.

B. *Bovine Tracheal Muscle.* This series was conducted as follows. For the experiments with adrenaline alone, two preparations were taken and mounted in glucose-free Tyrode's solution in the organ bath. In order to amplify the tonus, carbaminoylcholine $2.5 \cdot 10^{-7}$ (5 μg/20 ml) was added after 15 minutes. When the tonus had reached a constant level 10 minutes after this addition, adrenaline was added to one preparation in a concentration of $2 \cdot 10^{-6}$ (40 μg/20 ml), and 5 minutes later both preparations were taken for glycogen assay.

Adrenaline Experiments. The basal glycogen content of tracheal muscle showed a very substantial variation that was partly seasonal. After carbaminoylcholine the basal glycogen content in 51 adrenaline experiments averaged 270.4 mg per cent, the range being 130—422 mg per cent. After addition of adrenaline it showed a mean fall of 14.0 ± 4.2 mg per cent ($P < 0.001$). There was no demonstrable correlation between the effect of adrenaline and the magnitude of the basal glycogen content. Similarly, the fall was equally great in experiments conducted in June and July as in those performed in January and February. The effect of adrenaline, therefore, was

independent of the seasonal variation in the basal glycogen content.

In identical experiments on tracheal muscle in which, however, the lactic acid stimulating effect of adrenaline was determined, MOHME-LUNDHOLM (1953, 1956 a) found an increase of 21.6 ± 3.1 mg per cent in the lactic acid content, taking the average of 18 experiments. If the glycogen were quantitatively converted into lactic acid, then a decrease of 14.0 ± 4.2 mg per cent in the glycogen content should raise the lactic acid content by 15.5 ± 4.6 mg per cent. The difference between the observed and the expected increase of the lactic acid content, 6.1 ± 5.2 mg per cent, was not statistically verifiable.

Another series of experiments was conducted to find out if carbaminoylcholine influenced the glycogen content of tracheal muscle or the glycogenolytic effect of adrenaline. Four preparations were taken, to one of which was added carbaminoylcholine ($2.5 \cdot 10^{-7}$), to another adrenaline ($2 \cdot 10^{-6}$), and to a third both carbaminoylcholine and adrenaline; the fourth preparation served as a control. The preparations were removed for assay 20 minutes after the addition of carbaminoylcholine and 10 minutes after that of adrenaline. The results are shown in table II. Both adrenaline alone and carbaminoylcholine alone had a glycogenolytic effect. The effect of the two drugs in combination was somewhat greater than the totalled effect of each one separately, though not great enough to demonstrate statistically that carbaminoylcholine potentiated the effect of adrenaline. The result nevertheless showed that although adrenaline and carbaminoylcholine produced an antagonistic effect on muscle tonus, they had a synergistic action on the glycogenolysis.

Experiments with Glycolysis Inhibiting Substances. MOHME-LUNDHOLM (1953, 1956 b) observed that some substances which, in enzymological experiments, had been found to inhibit the enzymatic breakdown of glycogen and glucose into lactic acid, were also able to block the lactic acid producing and the relaxing effects of adrenaline. An investigation seemed worth while, to find out if these substances inhibited the glycogenolytic effect of adrenaline in the same concentration that blocked the above-mentioned effects.

The glycolysis inhibitors studied here were Cu^{++} ions, sodium fluoride, calcium ions, sodium azide and sodium arsenate. In the experiments with enzyme inhibitors, three contiguous prepara-

Table II.

The effect of adrenaline ($2 \cdot 10^{-6}$) and carbaminoylcholine ($2.5 \cdot 10^{-7}$) on the glycogen content of the bovine tracheal muscle.

Drug	Basal value mg per cent	Deviation from basal value after addition of drug mg per cent	Number of test	P
Adrenaline	320	-8.9 ± 9.7	15	0.4 — 0.3
Carbaminoylcholine..	320	-19.3 ± 7.75	15	0.05— 0.02
Carbaminoylcholine+ Adrenaline	320	-32.7 ± 7.87	15	< 0.001

tions were mounted in the organ bath. After the addition of carbaminoylcholine an interval of 10 minutes was allowed for the rise of tonus to reach its maximum, whereupon one of the enzyme inhibitors was added to two of the preparations. After a further 10 minutes adrenaline was added to one of these two treated preparations. Five minutes later all three preparations were taken for glycogen assay.

Cu^{++} Ions. These were added in the form of copper chloride in a concentration of 0.003 M (10 mg $CuCl_2 \cdot 2\,H_2O$/20 ml). In some experiments the copper chloride had a slight stimulating effect on the tonus. The relaxing effect of adrenaline was totally inhibited by the Cu^{++} ions in all 12 experiments. The effect of Cu^{++} ions and adrenaline on the glycogen content of tracheal muscle is shown in table III. The glycogen content was higher in the copper chloride preparations than in the controls. There was no demonstrable difference in glycogen content between preparations treated with copper chloride alone and those treated with adrenaline too. The Cu^{++} ions had accordingly inhibited the glycogenolytic effect of adrenaline, just as they inhibited its lactic acid producing and relaxing effects.

The rise in glycogen content under the influence of Cu^{++} ions was not due to synthesis of glycogen; rather it was attributed to inhibition of the spontaneous glycogenolysis occurring in the muscle. This was evident from a series of eight experiments in which the glycogen content was determined not only in the Cu-treated preparation but in two control preparations not treated

Table III.

The effect of glycolysis inhibiting drugs on the glycogenolytic effect of adrenaline ($2 \cdot 10^{-6}$).

Drug	Basal value mg per cent	Deviation from basal value mg per cent	Number of tests	P	Difference: (drug + adrenaline) — drug	P
Cu^{++} (0.003 M)	132	+ 32.6 ± 14.3	12	0.05—0.02	5.4 ± 15.1	0.8 —0.7
Cu^{++} + adrenaline		+ 37.1 ± 10.4	12	0.01—0.001		
F^- (0.016 M)	153	+ 17.6 ± 7.87	16	0.05—0.02	0.4 ± 6.71	0.6 —0.5
F^- + adrenaline		+ 17.9 ± 7.94	16	0.05—0.02		
Ca^{++} (0.15 M)	290	— 29.1 ± 7.26	16	0.01—0.001	— 0.3 ± 8.4	0.8 —0.7
Ca^{++} + adrenaline		— 28.8 ± 13.5	16	0.05—0.02		
NaN_3 (0.015 M)	239	— 56.5 ± 8.37	16	< 0.001	8.8 ± 11.1	0.5 —0.4
NaN_3 + adrenaline		— 47.7 ± 9.29	16	< 0.001		
Na_2AsO_4 (0.11 M)	242	+ 13.3 ± 10.6	18	0.3—0.2	— 8.5 ± 3.8	0.05—0.02
Na_2AsO_4 + adrenaline		+ 4.9 ± 9.38	18	0.7—0.6		

with carbaminoylcholine: the first of these was taken for assay coincident with the addition of copper chloride to the Cu preparation; the other and the Cu preparation were taken 15 minutes later. During this time the glycogen content of the second control preparation decreased in relation to the first by an average of 22 mg per cent. In the copper chloride preparation the glycogen content diminished in relation to the first control by an average of 9 mg per cent, but rose in relation to the second control by 13 mg per cent. The Cu^{++} ions thus partially inhibited the spontaneous glycogenolysis, and in relation to the second control this inhibition presented as an increase of the glycogen content.

Fluoride. Sodium fluoride was added in concentration of 0.016 M (13.5 mg/20 ml). It had a distinct stimulating effect on the tonus. Sodium fluoride totally blocked the relaxing effect of adrenaline in 11 of 16 cases; in the other five it had some effect, though greatly attenuated. Like copper ions, it inhibited the spontaneous glycogenolysis, as was evident from the apparent rise of 17.6 mg per cent in the glycogen content of the fluoride preparations by comparison with the controls. Taking the mean of all fluoride-adrenaline experiments, the glycogenolysis was inhibited to the same degree ($+$ 17.2 mg per cent) as in the fluoride experiments.

In the five cases where the relaxing effect of adrenaline was merely attenuated, adrenaline nevertheless seemed to have some glycogenolytic action. The difference between the fluoride and the fluoride-adrenaline experiments in these cases was -11.6 ± 9.0 mg per cent. The corresponding difference in those experiments where the relaxing effect of adrenaline was totally inhibited amounted to $+5.8 \pm 8.5$ mg per cent. Although the glycogenolytic effect of adrenaline was not statistically verifiable in cases with partial inhibition of the relaxing effect, it was nevertheless interesting, suggesting as it did that the threshold concentrations of sodium fluoride for total inhibition of the glycogenolytic and relaxing effects of adrenaline were equal.

Calcium^{++} Ions. Addition of calcium chloride in a concentration of 0.15 M (400 mg $CaCl_2 \cdot H_2O$/20 ml) had a stimulating effect on tracheal muscle tonus. It had a similar effect on the glycogenolysis, for the glycogen content of the Ca^{++} preparations fell by an average of 29.1 ± 7.3 mg per cent in relation to the controls. The Ca^{++} ions inhibited in each case the relaxing effect of adrenaline, and likewise its glycogenolytic action. Taking

the mean of 16 experiments, the glycogen content in the Ca^{++}-adrenaline preparations rose by 0.3 ± 8.4 mg per cent in relation to the Ca^{++} preparations. The Ca^{++} ions accordingly inhibited completely the relaxing and the glycogenolytic effect of adrenaline, although they stimulated glycogenolysis. That Ca^{++} ions also inhibit the lactic acid producing effect of adrenaline has been shown by MOHME-LUNDHOLM (1956 b).

The glycogenolytic effect of Ca^{++} ions had an interesting bearing on the discussion of the mechanism of action of Ca^{++} on the carbohydrate metabolism. According to BOYER, LARDER & PHILIPS (1943), Ca^{++} inhibits phosphopyruvic kinase, which catalyses the reaction phosphopyruvic acid + ADP $\rightleftharpoons$ pyruvic acid + ATP. The possibility existed, however, that Ca^{++} by precipitating phosphate could also inhibit the carbohydrate metabolism. Since Ca^{++} ions stimulate the breakdown of glycogen, which reaction is dependent on the presence of inorganic phosphate, this point of interference seems unlikely.

Sodium Azide. Sodium azide in a concentration of 0.015 M (20 mg/20 ml) had a slight relaxing effect on tracheal muscle. The spontaneous glycogenolysis was greatly stimulated. The difference between the azide experiments and the controls amounted to -56.5 ± 8.4 mg per cent. In the azide-adrenaline experiments the corresponding difference was -47.7 ± 9.3 mg per cent; *i. e.*, the glycogenolytic effect of adrenaline was totally inhibited. However, in five of the 16 experiments its relaxing effect was not fully blocked. In these cases adrenaline reduced the glycogen content by 15.6 ± 8.9 mg per cent in relation to the azide experiments. Where the relaxing effect was completely blocked, the difference between the azide-adrenaline and the azide experiments amounted to $+ 19.9 \pm 14.6$ mg per cent. The difference between the effects of adrenaline on the glycogen content in total and in partial inhibition of the relaxing effect (35.5 ± 17.1 mg per cent) was statistically probable ($P = 0.05$). As in the fluoride experiments, the threshold concentrations were equal for total inhibition of the relaxing and of the glycogenolytic effect.

Since both Ca^{++} ions and sodium azide inhibited the glycogenolytic action of adrenaline but concurrently stimulated the glycogenolysis, it seems unlikely that adrenaline's lactic acid producing effect resulted from its activation of phosphorylase.

It was possible, by adding glucose, partially to abolish the

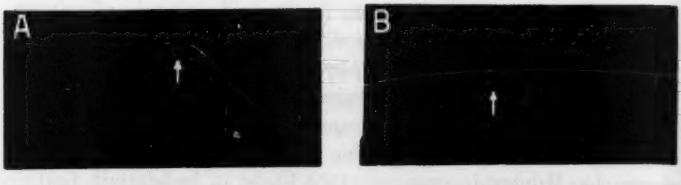

Fig. 1. Bovine tracheal muscle. Blocking by sodium azide of the relaxing effect of adrenaline was abolished by glucose. A. At the arrow, 40 μg adrenaline per 20 ml. B, C and D. The same dose of adrenaline, but 10 mg sodium azide per 20 ml had been added 10 minutes earlier. In B the glucose content was 0 per cent; in C, 0.5 per cent; and in D, 1 per cent.

blocking by sodium azide of adrenaline's relaxing effect on tracheal muscle. Fig. 1 shows that 0.0075 M sodium azide totally blocked the relaxing effect of adrenaline, while addition of 0.5 and of 1 per cent glucose gradually eliminated this blockade.

Sodium Arsenate. Arsenate was added in a concentration of 0.11 M (400 mg Na_2HaSO_4/20 ml). The arsenate solution was adjusted to pH 6.9 with sodium hydroxide. Sodium arsenate had a relaxing effect on tracheal muscle; and in order to avoid excessive relaxation, making the effect of adrenaline more difficult to evaluate, the arsenate was added in a dose of 100 mg at 2 minute intervals.

Sodium arsenate probably inhibited the spontaneous glycogenolysis, since the difference between the arsenate and control experiments was $+ 13.3 \pm 10.6$ mg per cent. The relaxing effect of adrenaline was totally abolished only in 9 of the 18 experiments, and produced there a fall of 7.4 ± 5.0 mg per cent in the glycogen content. In the nine experiments with only a partial blocking effect on the tonus, the glycogen content decreased by 9.6 ± 6.1 mg per cent. Taking the mean of all experiments, adrenaline reduced the glycogen content by 8.5 ± 3.8 mg per cent, which fall was statistically probable. The effect of adrenaline on the lactic acid production of muscle treated with arsenate varied

in this respect. Only in those cases where the relaxing effect of adrenaline was attenuated could lactic acid production be demonstrated. With total abolition of its relaxing effect, the lactic acid producing effect was also blocked (MOHME-LUNDHOLM 1953).

C. *Bovine Coronaries.* The experimental procedure was as follows. Two adjacent specimens of artery were mounted in the organ bath in Tyrode's solution containing no glucose. When a satisfactory spontaneous tonus had been reached after 10—15 minutes, adrenaline was added to one preparation in a concentration of $1 \cdot 10^{-6}$ (20 μg/ml). The relaxing effect — sometimes preceded by very slight contraction — began after about 30 seconds and reached a maximum after approximately 5 minutes, when the preparations were taken for assay.

The basal glycogen content averaged 187 mg per cent, with a range of 87—390 mg per cent. The glycogen content fell by 3.7 ± 6.1 mg per cent, taking the mean of 30 experiments. The fall, accordingly, was not statistically verifiable.

In similar experiments in which the increase of lactic acid was instead determined, MOHME-LUNDHOLM (1957) found that the lactic acid content rose by 5.1 ± 0.85 mg per cent, taking the average of 22 experiments. The amount of lactic acid that was calculated to result from the glycogenolysis — 4.1 mg per cent — thus closely accorded with that found experimentally.

Guinea-Pig Uterus. These experiments were conducted on a total of 21 uteri, chiefly from mature guinea-pigs. One uterine horn served as a control, and to the other was added adrenaline $1 \cdot 10^{-6}$. Five minutes after addition of adrenaline, both horns were taken for assay. The basal glycogen content averaged 183 mg per cent, the range being 110—357 mg per cent. After adrenaline the glycogen content fell by a mean figure of 7.3 ± 9.4 mg per cent. However, in only 12 experiments did adrenaline have an unequivocal relaxing and activity-inhibiting effect. In these experiments the glycogen content fell by an average of 27.4 ± 12.1 mg per cent; *i. e.*, the fall was statistically probable ($0.05 > P > 0.02$). In the remaining nine experiments, in which adrenaline had a very slight or indefinite effect, the glycogen content *increased* by an average of 19.6 ± 9.1 mg per cent.

In a similar series of 13 experiments on guinea-pigs, most of which were immature, MOHME-LUNDHOLM (1953) found a mean increase of 32.6 ± 7.6 mg per cent in the lactic acid content. Since in that series adrenaline consistently had a distinct relaxing

effect, this rise of lactic acid should be compared with the glycogen reduction of 27.4 mg per cent in the series where adrenaline had a conspicuous relaxing effect. The lactic acid value equivalent to this glycogen figure was 30.1 ± 13.3 mg per cent, so that the decrease of glycogen closely accorded with the expected increase of the lactic acid content.

HOLTZ and WÖLLPERT (1937), in experiments on guinea-pig uterus, found that the relaxing effect of adrenaline changed to a stimulating action during estrus and at the beginning and end of pregnancy. A similar alteration of the effect was also obtained when virgin guinea-pigs were treated with gonadotrophic hormone or follicular hormone.

In our experiments we found no stimulating effect of adrenaline but only a loss of the relaxing effect; however, this divergence from HOLTZ and WÖLLPERT's results may well be attributable to differences in composition of the nutrient solutions. Our experiments were conducted with a neutral (pH 7.1) Tyrode solution containing no glucose, whereas HOLTZ and WÖLLPERT employed a more alkaline Locke-Ringer solution containing 0.5 per cent glucose. Both a high glucose content (unpublished experiments) and an alkaline environment (EVANS and UNDERHILL 1923) amplify the stimulating effect of adrenaline on smooth muscle.

The difference in the effect of adrenaline on the glycogen content between the experiments in which it had a relaxing effect on uterus and those where no distinct effect was obtained, amounted to 47.0 ± 15.2 mg per cent, which is highly probable ($0.01 > P > 0.001$). It is worth while, in this connection, comparing adrenaline's effects on glycogenolysis in bovine coronaries and mesenteric vessels, the former of which it relaxes and the latter it stimulates. In 21 experiments on mesenteric vessels with a technique identical to that used for coronaries, the glycogen content rose by an average of 11.9 ± 4.9 mg per cent (LUNDHOLM and MOHME-LUNDHOLM, to be published). The difference of 15.6 ± 7.8 mg per cent between adrenaline's effects on the glycogen content of mesenteric and of coronary vessels was statistically probable ($P = 0.05$). Further experiments showed that in mesenteric vessels adrenaline did not give rise to synthesis of glycogen but instead reduced the velocity of spontaneous glycogenolysis. The effect of adrenaline on the glycogen content of uteri probably has a similar explanation. The relaxing effect

on smooth muscle is thus associated with increased glycogenolysis, whereas the stimulating effect retards the glycogenolysis.

Discussion.

MOHME-LUNDHOLM (1953, 1956 a, 1957) found that adrenaline and other sympathomimetic amines exerted their relaxing effect on smooth muscle by increasing the lactic acid production in that tissue. In the experiments reported above we observed that the lactic acid production and the relaxing effect after adrenaline were associated with an increase of glycogenolysis. In tracheal muscle, coronary arteries and uterus the glycogenolysis and lactic acid production were of the same order of magnitude, whereas in rabbit intestine the former was substantially less than the latter. Various glycolysis inhibiting substances that blocked the relaxing and lactic acid producing effects of adrenaline also inhibited the glycogenolytic effect, while in partial blocking of the relaxing effect the same applied to the glycogenolytic effect. These results accordingly demonstrate the intimate relationship between the glycogenolytic effect of adrenaline on the one hand and its relaxing and lactic acid producing effects on the other.

The question whether the glycogenolytic action was the primary cause of the relaxing and lactic acid producing effects — *i. e.*, whether adrenaline's effect on the carbohydrate metabolism can be fully attributed to its activation of the enzyme phosphorylase — must, however, be answered in the negative. If glycogenolysis were the primary reaction, then the glycogen loss should logically be greater than the lactic acid production. The glycogen loss was compared with the increase of the lactic acid content in the muscle, no account being taken of the lactic acid that diffused into the Tyrode solution. In the Embden-Meyerhof scheme of glycolysis there are, moreover, at least eleven intermediate products between glycogen and lactic acid. Since there is a certain measure of equilibrium between these intermediates, it is plausible to assume that in glycogenolysis leading to an increased lactic acid production a substantial part of the lost glycogen would be converted to those products. Some substances, such as Ca^{++} ions and sodium azide, which themselves had a glycogenolytic effect, at the same time inhibited the lactic acid producing and glycolytic effects of adrenaline; and this finding would be difficult to explain if adrenaline's sole point of interference in the carbohydrate metabolism were phosphorylase.

Moreover, glucose both inhibited the glycogenolytic effect of adrenaline on rabbit gut and also abolished the blocking action of sodium azide on adrenaline's relaxing effect. This suggests, too, that adrenaline influences some reaction in the carbohydrate metabolism independently of its activating effect on phosphorylase.

Further support for the view that activation of phosphorylase is only one of adrenaline's points of attack in the carbohydrate metabolism, lies in RIESSER's (1947) report that under conditions in which isolated rat diaphragm synthesized glycogen, adrenaline induced glycogenolysis. SUTHERLAND (1952), in experiments in isolated rat diaphragm, found that 8-hydroxyquinoline had an adrenaline-like effect on the carbohydrate metabolism of the muscle, since it produced glycogenolysis, increased lactic acid production and decreased glucose absorption. However, when combined with 8-hydroxyquinoline, adrenaline produced instead an increased glucose absorption concurrently with a fall in the content of glucose-6-phosphate. Without supplementary 8-hydroxyquinoline, it elevated that content. SUTHERLAND assumed this to imply that adrenaline had an effect on the carbohydrate metabolism which was not dependent on activation of phosphorylase.

ELLIS, ANDERSON and MCGILL (1955) found that the activation of phosphorylase by adrenaline in liver slices was a process, requiring energy, that did not occur under anaerobic conditions and that was inhibited by 2,4-dinitrophenol. This finding accords with the observation of FISCHER and KREBS (1955) that the conversion of phosphorylase *b* to *a* requires the presence of ATP. In contrast to this state of affairs we found, in experiments on bovine mesenteric vessels, that under anaerobic conditions adrenaline increased the tonus and stimulated the lactic acid production. Dinitrophenol blocked the former but not the latter effect. (LUNDHOLM and MOHME-LUNDHOLM, to be published.)

All of these facts suggest, therefore, that adrenaline not only has its activating effect on phosphorylase but a further point of interference in the carbohydrate metabolism. Where this is to be sought cannot even be surmised at present. Since adrenaline under some conditions may reduce the content of glucose-6-phosphate on coincident stimulation of the glucose absorption, this effect is localized somewhere in the reaction chain, glucose-6-phosphate → lactic acid. But since this reaction has ten intermediates, there are still numerous conceivable points of attack

for adrenaline. The possibility should also be taken into account that adrenaline influences the carbohydrate metabolism secondarily, *e. g.* via an increased ATP metabolism.

Summary.

The effect of adrenaline on the glycogen metabolism of smooth muscle was investigated in experiments on rabbit gut, bovine tracheal muscle and coronary arteries, and guinea-pig uterus. The glycogenolytic effect was correlated with the lactic acid stimulating and the relaxing effects.

On rabbit gut, adrenaline had only a glycogenolytic effect in the absence of glucose. The decrease of glycogen was substantially less than the equivalent increase in the lactic acid content, and occurred later than the lactic acid production.

In experiments on tracheal muscle adrenaline had a glycogenolytic effect of the same order of magnitude as the lactic acid production. Adrenaline and carbaminoylcholine had a synergistic glycogenolytic action, but an antagonistic effect on the tonus.

Glycolysis inhibiting substances such as Cu^{++}, Ca^{++}, NaF, NaN_3 and Na_2HAsO_4, which inhibited the lactic acid producing and the relaxing effects of adrenaline, similarly depressed its glycogenolytic action on tracheal muscle. With partial inhibition of the relaxing effect, the same applied to the glycogenolytic one too.

Calcium ions and sodium azide had a stimulating effect on the glycogenolysis, but other glycolysis inhibiting drugs reduced the spontaneous glycogenolysis.

Glucose abolished the blocking action of sodium azide on adrenaline's relaxing effect.

In coronary arteries adrenaline produced glycogenolysis equivalent to the lactic acid production.

On guinea-pig uterus adrenaline had only a glycogenolytic effect in the experiments where distinct inhibition of the tonus or pendulum movements was obtained; in those where the relaxing effect was absent, the glycogen content rose in relation to the controls.

Cori and Sutherland (1951) showed that adrenaline stimulates glycogenolysis in liver tissue and striated muscle by activating the enzyme phosphorylase, which catalyzes the reaction glycogen + phosphoric acid $\rightleftharpoons$ glucose-1-phosphate. The effect

of adrenaline on the glycogenolysis and lactic acid production in smooth muscle cannot be attributed solely to activation of this reaction; rather, adrenaline probably has at least one further point of interference in the carbohydrate metabolism.

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Mechanism of the Relaxing Effect of Adrenaline on Bovine Coronary Vessels.

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In earlier investigations I found that the relaxing effect of adrenaline and other sympathomimetic drugs on smooth muscle was due to the fact that they increased the lactic acid content of smooth-muscle cells. These experiments were conducted on unstriped muscle from the alimentary, respiratory and genitourinary tracts (MOHME-LUNDHOLM 1953, 1956 a). However, adrenaline in low concentrations also has a vasodilator effect in certain vascular areas, as in skeletal muscle, the liver and the heart. In skeletal muscle it produces vasodilatation only when injected intravenously or intra-arterially. Isolated skeletal muscle vessels are constricted by adrenaline in all active concentrations (ROTHLIN 1920, VON TAFEL 1931). This paradox has been attributed to the fact that adrenaline induces lactic acid production in the skeletal muscle cells which gives rise to its dilator effect in that vascular area (LUNDHOLM 1957). The only isolated blood vessel that is demonstrably relaxed by adrenaline is the bovine coronary (BARBOUR 1912 a, ROTHLIN 1920, CRUICKSHANK and SUBBA RAU 1927, DUCRET 1930 a, COHEN 1936, SMITH 1950). Coronary vessels from swine, too, are in most cases dilated by adrenaline, though constriction may occur (SMITH 1950). In man (BARBOUR 1912 b, SMITH 1950), monkey (BARBOUR 1912 b) and horse (ROTHLIN 1920) they are constricted. The relaxation produced by adrenaline in some experiments on renal arteries (ROTHLIN 1920) and pulmonary arteries (WISSLER 1931, CHANG 1932) may be due to the presence of both circular and longitudinal fibers

in those vessels. — With contraction of the longitudinal fibers there is a lengthening of the circular fibers, manifested in relaxation (FURCHGOTT 1955, LUNDHOLM 1957).

With the aim of investigating the possible relationship between the relaxing and the lactic acid producing effect of adrenaline on vascular muscle, I therefore conducted experiments on coronary vessels from cow. The corresponding effects of noradrenaline were studied too.

Method.

Coronary arteries were taken from cows 10—15 minutes after slaughter. A sound was introduced into the coronaries from the aorta, after which they were dissected free and denuded of fat and muscle. The vessels were then immersed in iced Tyrode's solution. Both the right and left arteries were taken for the experiments, but only segments with calibers of at least 3—4 mm were used. The portions adjacent to the aorta had too much elastic tissue to be suitable for recording of tonus changes in the smooth muscle layer. For the experiments, specimens were taken about 5 mm in length; they were cut longitudinally so as to form rectangular pieces with a length of 10—15 mm and a width of 5 mm. Two adjacent segments were used, one of them as a control and the other being treated with adrenaline, noradrenaline or lactic acid. The weight of these preparations ranged from 0.1 to 0.4 g.

The specimens were immersed in an organ bath *ad modum* Magnus. The tonus variations were registered with an isotonic recorder having a load of 2 g. In these experiments it was found appropriate to use a Tyrode solution with weaker buffering than normal. The content of bicarbonate and phosphate was reduced to one-fourth, so that the solution had the following composition: 0.8 % NaCl; 0.02 % KCl; 0.02 % $MgCl_2$; 6 H_2O; 0.025 % $NaHCO_4$; 0.00125 % NaH_2PO_4; 2 % H_2O, and 0.1 % glucose.

The reason for reducing the buffering capacity of the Tyrode solution here was as follows. In order that the higher lactic acid content produced in the smooth muscle by the supplementary lactic acid would fully correspond to the increase after adrenaline, equivalent amounts of lactate ions and hydrogen ions had to diffuse in when the lactic acid was added. If a relatively small amount of lactic acid (10 mg/20 ml) was added to normally buffered Tyrode's solution, the latter's pH was substantially reduced for the first two minutes (MOHME-LUNDHOLM 1953). During this time the conditions were fairly conducive to the diffusion of equivalent amounts of lactate ions and hydrogen ions into the cells; for at pH 4, lactic acid shows a dissociation of only 65 per cent, whereas at pH 7 the degree of dissociation is 99.9 per cent. FURCHGOTT and WALES (1951, 1952) found that an organic acid penetrates into smooth muscle more readily in undissociated than in dissociated form. The initially reduced pH of the Tyrode solution is,

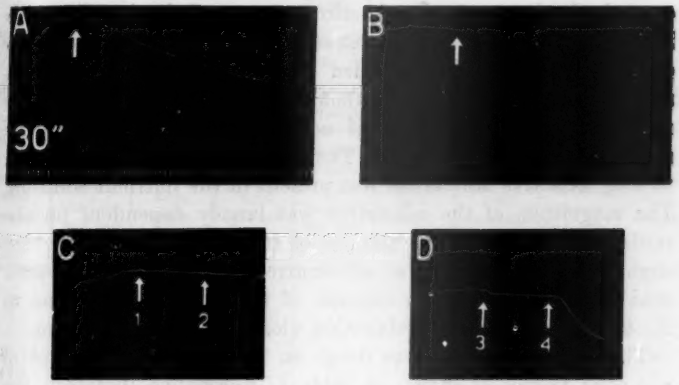

Fig. 1. Bovine coronary vessels.

A. Adrenaline in a concentration of $1 \cdot 10^{-6}$.
B. Lactic acid, conc. $5 \cdot 10^{-4}$.
C. 1. $CuCl_2$, conc. 0.003 M.
2. 10 min. later, adrenaline conc. $1 \cdot 10^{-6}$.
D. 3. $CuCl_2$, conc. 0.003 M.
4. 10 min. later, theophyllamin conc. $2 \cdot 10^{-3}$.

however, transient, since the carbonic acid liberated from bicarbonate gradually breaks down into carbon dioxide and water. With a rising pH the probability increases that lactate ions will instead diffuse into the cells together with potassium ions.

The coronary arteries reacted with a gradual fall of tonus both after adrenaline and after lactic acid (fig. 1). This fall did not reach its maximum until more than five minutes had elapsed. In order to ensure that throughout this time lactic acid would diffuse into the cells largely in undissociated form, the buffering capacity of the Tyrode solution had to be so reduced that the supplementary lactic acid produced a constant fall of the pH.

The lactic acid content of the tissues was determined by a method that has earlier been described in detail (MOHME-LUNDHOLM 1953).

Results.

In the experiments on coronary vessels two adjacent segments were taken and immersed in the organ bath. The tonus increased rapidly and reached a maximum after 10—15 minutes, after which it fell again and returned to the initial value after about 30 minutes. To one specimen was added, when the tonus had reached its maximum, adrenaline in a concentration of 10^{-6} (20 μg/20 ml) or noradrenaline in the same concentration, or lactic acid in a concentration of $5 \cdot 10^{-4}$ (10 mg/20 ml). In most

cases both the sympathomimetic amines and lactic acid were initially followed by slight contraction lasting about one-half to one minute. This was succeeded by the relaxing effect, which reached its maximum after about 5 minutes (fig. 1 A). Five minutes after the addition of adrenaline the specimens were removed for lactic acid assay. The relaxation otherwise persisted as long as active adrenaline was present in the nutrient solution. The magnitude of the relaxation was largely dependent on the initial tonus. When the initial rise of tonus was small, only very slight relaxation or none at all occurred. In such cases no lactic acid assay was done. An increase of the dose of adrenaline to 2—400 μg/ml produced relaxation alone and no contraction.

The effect of the various drugs on the lactic acid content of coronary vessels is shown in table I. Adrenaline increased the lactic acid content by 5.1 mg per cent, taking the average of 22 experiments; *i. e.* by 31 per cent of the initial value. After noradrenaline the corresponding rises were 3.6 mg per cent and 22 per cent of the initial value, taking the average of 38 experiments. In order to secure a measure of the relaxing effect, the angle formed by the tonus curve after addition of the various drugs was measured. This angle averaged 27 degrees in the adrenaline experiments and 26 degrees in the noradrenaline experiments. These two drugs thus showed approximately the same activity in experiments on bovine coronary vessels. In similar experiments on coronary vessels from swine, Smith and Coxe (1950) found noradrenaline to be two and a half times more active than adrenaline.

Lactic acid was added in a concentration of $5 \cdot 10^{-4}$ (10 mg/20 ml), which dose regularly had a distinct relaxing effect when the initial tonus was good (fig. 1 B). Even half of this dose produced a relaxing effect, but since the latter did not occur in all cases the higher dose was used throughout. The entire amount of lactic acid, it may be pointed out, should be added in a single dose if maximum relaxation is to be obtained. When the dose was divided into two equal parts with an interval of a few minutes, only slight relaxation or none at all was observed.

Taking the mean of 29 experiments a relaxation angle of 43 degrees and a rise of 12 per cent in the lactic acid content were found. The supplementary lactic acid thus produced both a greater increase in the lactic acid content and somewhat greater relaxation than did adrenaline or noradrenaline. However, the relevant

Table I.

Effect of l-adrenaline, l-noradrenaline and dl-lactic acid on tone and lactic acid content of isolated bovine coronary arteries.

Adrenaline $1 \cdot 10^{-6}$			Noradrenaline $1 \cdot 10^{-6}$			Lactic acid $5 \cdot 10^{-4}$		
Basal value lactic acid content mg per cent	Increase of lactic acid content mg per cent	Relaxation angle	Basal value lactic acid content mg per cent	Increase of lactic acid content mg per cent	Relaxation angle	Basal value lactic acid content mg per cent	Increase of lactic acid content mg per cent	Relaxation angle
19.7	2.0	55	8.2	10.7	20	10.8	17.0	29
21.3	4.8	11	8.6	7.5	25	16.6	8.7	30
15.1	3.2	26	14.6	— 6.7	21	13.2	16.7	90
16.2	1.0	13	22.0	2.5	18	12.3	15.9	46
14.1	5.2	10	8.2	5.0	18	14.9	17.1	100
7.5	13.7	46	3.7	— 2.0	40	10.7	10.5	72
16.6	1.9	76	3.2	6.6	8	12.0	14.3	16
16.7	8.3	57	21.7	8.5	49	12.1	13.2	23
15.5	3.9	26	20.0	0.8	17	11.2	11.2	47
15.8	4.5	6	25.7	— 2.6	43	10.6	13.2	11
18.1	8.3	30	25.1	— 0.5	0	9.8	16.2	60
14.6	4.9	1	22.2	4.0	10	25.3	15.9	67
14.6	6.9	26	21.5	7.9	38	18.5	5.8	14
15.5	11.6	55	22.2	4.4	10	15.8	14.8	12
13.1	9.5	23	21.5	7.4	28	12.5	7.5	56
16.4	0.4	25	22.0	7.8	13	14.3	15.0	64
27.2	6.7	25	19.7	— 2.9	44	17.0	9.1	78
33.5	— 3.8	19	21.3	1.9	15	14.3	9.3	39
15.9	2.2	11	15.1	— 0.2	8	22.0	3.9	70
17.1	2.7	27	16.2	2.2	22	15.4	13.5	33
8.0	8.9	18	14.1	5.3	8	17.9	14.4	31
14.6	5.0	0	7.5	3.0	57	10.4	10.3	25
			16.6	1.0	30	14.6	15.8	71
			16.7	14.8	53	12.2	7.2	57
			15.5	15.0	20	17.6	3.8	29
			15.8	9.6	36	15.8	3.1	38
			18.1	3.0	5	14.4	15.7	25
			14.6	6.7	62	13.6	13.8	8
			14.6	7.3	28	14.6	14.6	10
			15.5	5.6	74			
			13.1	6.3	37			
			16.4	1.4	45			
			27.2	— 8.6	0			
			33.5	— 9.0	35			
			15.9	— 0.1	18			
			17.1	3.1	12			
			8.0	6.3	8			
			14.6	3.8	17			
Mean								
16.7	5.1	26.6	16.3	3.6	26.1	14.5	12.0	43.1
S. E. of the mean	± 0.85	± 4.2		± 0.88	± 2.9		± 0.80	± 4.8
P	< 0.001	< 0.001		< 0.001	< 0.001		< 0.001	< 0.001

increase that occurred in the adrenaline and noradrenaline experiments was not directly comparable with that in the lactic acid experiments, since under basal conditions and under the influence of adrenaline or noradrenaline the lactic acid and the lactic acid production respectively are probably localized to the smooth muscle layer. DUCRET (1930 b), in histologic measurements, found that about 60 per cent of bovine coronary artery wall consisted of media and that 45—65 per cent of the latter was composed of smooth muscle, the remainder being connective and elastic tissue. Smooth muscle thus accounted for 30—40 per cent of the coronary wall.

After addition of adrenaline the lactic acid content of the *entire* wall rose by 5.1 mg per cent. The lactic acid production, however, was probably confined to the smooth muscle layer, which averaged about 35 per cent of the vessel wall. The actual increase within the smooth-muscle cells may therefore be estimated at 14.6 mg per cent in the adrenaline experiments and 10.3 mg per cent in those with noradrenaline; *i. e.*, an average of 12.5 mg per cent, which figure accords with the increase after addition of lactic acid.

With an increase in the lactic acid content of the smooth muscle, some lactic acid naturally diffuses into the extracellular fluid, thereby increasing the total lactic acid content of the vessel; but the increase there is probably minimal, since in smooth muscle lactic acid by no means behaves like a freely diffusable substance. Even when equilibrium has occurred, the lactic acid content of the surrounding nutrient solution is still only 5 per cent of that in the vessel (LUNDHOLM and MOHME-LUNDHOLM, unpublished observation). On addition of lactic acid to the solution, the lactic acid is probably distributed approximately uniformly in the various tissues of the vessel. These calculations indicate that the average increase in the lactic acid content of the smooth muscle was, in the adrenaline and noradrenaline experiments (12.5 mg per cent), of the same order of magnitude as that after addition of lactic acid.

For a further study of the relation between the lactic acid producing and the relaxing effect of adrenaline, experiments were conducted with glycolysis inhibiting substances which had earlier (MOHME-LUNDHOLM 1953, 1956 b) been found to inhibit each of those two effects. Cu^{++} ions in a concentration of 0.003 M (10 mg $CuCl_2 \cdot 2\ H_2O$/20 ml) were added to two specimens mounted

Table II.

Effect of adrenaline and Cu^{++}-ions (0.003 M) on lactic acid content of isolated bovine coronary arteries.

	Basal value lactic acid content, mg per cent	Cu tests Lactic acid deviation from basal value, mg per cent	Cu-adrenaline tests Lactic acid deviation from basal value, mg per cent
	31.7	— 7.6	— 1.5
	22.5	— 1.1	— 1.1
	16.2	— 0.7	— 0.7
	18.4	2.8	2.8
	15.4	2.9	— 2.5
	18.3	— 3.2	— 2.0
	19.9	0.7	1.7
Mean	20.3	-0.89 ± 1.39	-0.47 ± 0.75
		$t=0.64$ $0.6>P>0.5$	$t=0.63$ $0.6>P>0.5$

in the organ bath. To one of these specimens adrenaline was then added in a concentration of $5 \cdot 10^{-7}$, 10 minutes after the addition of Cu ions. Both specimens were taken for lactic acid assay 5 minutes later. The relaxing effect of adrenaline was totally inhibited in all cases and, as will be seen from table II, the same also applied to the lactic acid producing effect. Other glycolysis-inhibiting substances such as sodium fluoride, monoiodoacetic acid, sodium azide, calcium ions, dl-glyceraldehyde, and sodium arsenate also totally inhibited the relaxing effect of adrenaline on coronary vessels. This effect was abolished, too, by alkalization of the Tyrode solution with sodium bicarbonate in a concentration of 1 : 100.

It is interesting in this connection to observe that the blocking, by glycolysis inhibiting substances and sodium bicarbonate, of the relaxing effect of the sympathomimetic amines is specific for those spasmolytics and does not apply to others such as theophylline, papaverine, and nitrites. From fig. 1 C, D it will be seen that copper ions in a concentration that totally inhibited the relaxing effect of adrenaline did not influence the spasmolytic effect of theophylline.

Discussion.

In vascular muscle as in other smooth muscle, the relaxing effects of adrenaline and noradrenaline are united and dependent

upon the stimulating action on the lactic acid production. This latter action, however, is not necessarily coupled with a relaxing effect. In mesenteric vessels from cow, on which adrenaline has a tonus-increasing effect, it also induces lactic acid production (SÜDHOF 1950, LUNDHOLM and MOHME-LUNDHOLM, unpublished observation). On the other hand, the glycogen metabolism of coronary and of mesenteric vessels is differently influenced by adrenaline. Whereas adrenaline tended to stimulate the spontaneous glycogenolysis in coronaries, it inhibited it in mesenteric vessels (LUNDHOLM and MOHME-LUNDHOLM 1957).

The significance to the blood flow through the heart, of the lactic acid producing and the relaxing effect of adrenaline in the coronaries is difficult to judge. WÊGRIA (1951), who discussed exhaustively the various factors leading to an increased coronary circulation after adrenaline, assumed that the principal one was the stimulating effect on the myocardium, with a consequent increase of metabolic products having dilator characteristics. Other factors of secondary importance were said to be the rise in perfusion pressure due to the increase of blood pressure, and the direct relaxing effect on the muscle layer. In this respect it is at present difficult from experimental evidence to judge the importance to the coronary circulation of the direct vasodilator effect of adrenaline. Up to now, the effect of adrenaline has been investigated only on isolated coronaries from large animals such as the cow, horse, sheep, swine and monkey, while the coronary blood flow under physiological conditions has been studied only in smaller animals such as the cat and dog, where technical difficulties have handicapped a study of the response of isolated coronaries to adrenaline. However, ECKENHOFF, HAFKENSCHIEL and LANDMESSER (1947), in experiments on dogs, found that adrenaline injected into the coronary artery produced a rise in the coronary blood flow even after doses small enough to have no stimulative effect on the myocardium. LOCHNER, MERCHER and SCHÜRMEYER (1956) observed, likewise in dogs, that adrenaline injected intravenously in doses that raised the blood pressure and pulse only very slightly, substantially reduced the arteriovenous oxygen difference in the coronary blood by elevating the oxygen content of the venous blood. These experiments suggest that, after small doses at least, the direct dilator effect of adrenaline on the coronaries is the most important of those factors which raise the coronary blood flow. Yet another factor, however,

conceivably has some significance in this dilator action of adrenaline on coronaries. As in striated and smooth muscle, adrenaline has a glycogenolytic (CHANG 1936) and lactic acid producing (GOTTDENKER and MARCHI 1937) effect on the heart muscle too. It seems plausible to assume that this stimulation of the lactic acid production, like that in striated muscle (LUNDHOLM 1956), is associated with vasodilatation. Whether the stimulating effect of adrenaline on glycolysis is merely a phenomenon accompanying its similar action on the myocardium, or is independent thereof, remains to be established, however.

Summary.

The effect of adrenaline, noradrenaline and lactic acid was investigated on isolated coronary vessels from cow.

All these substances had a relaxing effect, coincident with an increase in the lactic acid content of the smooth muscle. The respective effects produced by adrenaline and noradrenaline were of similar magnitude to those induced by addition of lactic acid.

Cu^{++} ions totally inhibited the relaxing and lactic-acid-producing effects of adrenaline and noradrenaline on coronary vessels. The former effect was also completely abolished by other glycolysis inhibiting substances such as monoiodoacetic acid, sodium fluoride, dl-glyceraldehyde, sodium azide, sodium arsenate and $Ca^{\cdot\cdot}$ ions, as well as by an increase in the alkalinity of the nutrient solution.

The effect of other spasmolytics, such as theophylline, was not inhibited by glycolysis inhibiting substances or alkalization of the nutrient solution.

These results suggest that the relaxing effect of adrenaline and noradrenaline on isolated blood vessels is due to their ability to stimulate the lactic acid production in the muscle layer.

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The Inhibitory Action of a Compound Obtained from Hip Seeds (HSC) on the Release of Histamine and the Disruption of Mast Cells Produced by Compound 48/80 and Extracts from Jellyfish (Cyanea Capillata) and Eelworm of Swine (Ascaris Lumbricoides).

By

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During the course of experiments on biologically occurring histamine releasers we investigated the action of extracts from hip seeds. These seeds are covered with hairs and produce itching on contact with the skin. We surmised that the itching might be due not only to the mechanical irritation but also to the presence of some histamine liberating substance — as reported by BROADBENT (1953) for Cowhage. However, extracts from the hip seeds did not produce any histamine release. On the contrary, they blocked the action of subsequently administered compound 48/80. This observation prompted us to examine further the inhibitory mechanism and the factor responsible for it. Although these investigations are still in progress, the observation of the inhibitory action of hip seed extracts seems to be worth a brief communication.

Technique.

The inhibitory action on histamine liberation was studied in the perfused paw of the cat. The paw was prepared and perfused with Tyrode's solution as reported by HÖGBERG, THUFVESSON and UVNÄS (1956).

The perfusion fluid was tested on guinea-pig ileum in 2 ml aerated Tyrode's solution containing atropine sulphate 1 μg/ml at 39° C. Histamine values are calculated as histamine base.

The identity with histamine of the active substance in the perfusates was confirmed by the complete abolition of its effect on guinea-pig neoanthergane (Anthisane) in concentrations of 10^{-6}—10^{-7}.

Preparation of Hip Seed Extracts.

Method I. Precipitation with Alcohol.

Hip seeds were obtained from fresh ripe hips. They were dried at room temperature for some weeks; then extracted in an approximately equal volume of 47 per cent ethyl alcohol for 60 minutes at 65° C. The alcohol was filtered off and the extraction repeated with a new portion of alcohol. The two portions of alcohol were then concentrated *in vacuo* on the water bath at 55° C to about one-fourth of the volume. Absolute alcohol was added, to a final concentration of 67 per cent. The gelatinous precipitate was centrifuged off and the remaining solution evaporated until foaming became too inconvenient. This meant that the volume was reduced to about 1/10—1/20 of the original volume. Absolute alcohol was then added until precipitation began and thereafter in great excess (to a final concentration of about 92 per cent). After centrifugation the somewhat gelatinous precipitate was dissolved in a few milliliters of water, the undissolved residue centrifuged off and discarded, and absolute alcohol again added to the water solution in great excess. A new precipitate formed. The precipitation with absolute alcohol was repeated until the precipitate obtained consisted of a white-yellow powder which could be dried *in vacuo* and was readily soluble in water.

Method II. Trichloracetic Acid Extraction.

An active hip seed extract was also obtained from air-dried ripe hips by pulverising the seeds in a mortar and then in a Waring blendor with iced trichloracetic acid (TCA). Starting with 60 g dried material, the first extraction was done with 250 ml iced TCA (10 per cent). After filtration the residue was re-extracted with an additional 125 ml TCA (5 per cent). The extracts were combined and cooled to 0° C. The pH was rapidly adjusted to 8.2 with 2 N NaOH and a great excess of barium was added in the form of barium acetate (25 per cent). The mixture was kept at 0° for 30 minutes. The resulting precipitate was removed by centrifugation and washed with barium acetate (1 per cent), alcohol (50 per cent), alcohol (absolute) and ether; then dried *in vacuo* over phosphorus pentoxide. For use, the barium salt was converted to the free acid with Dowex-50 resin in hydrogen form.

The above two methods yielded preparations having the same blocking activity on the disruption of mast cells.

Extracts from the jellyfish (*Cyanea capillata*) and from the eelworm of swine (*Ascaris lumbricoides*) were prepared as described by HÖGBERG, THUFVESSON and UVNÄS (1956).

Technique for Observations on the Distruption of Mast Cells.

A slight modification of the technique described by NORTON (1954) was used. The rat was killed by a blow in its head and the abdomen immediately opened. Suitable pieces of the mesentery were removed and placed in solutions of the substances to be tested. These substances had been dissolved beforehand in 5 ml portions of Tyrode's solution. After 20 minutes on the water bath at 37° C, the specimens of mesentery were transferred to another bath for fixation and staining. This bath contained 3.8 per cent formaldehyde and 0.1 per cent toluidine blue. Twenty minutes later the pieces were washed twice in acetone and twice in xylol. Each piece was denuded of fat and blood vessels; then mounted on a slide. One hundred mast cells were counted at random in four or five different parts of the preparation, and the percentage of ruptured cells was determined. The substance to be tested with respect to its inhibitory action was added before the addition of compound 48/80.

Results.

Figure 1 shows the inhibitory action of 1 mg HSC on the histamine liberation produced in the cat's paw by 10 μg 48/80. In the control paw 10 μg 48/80 liberated 34 μg histamine (*A* in fig. 1.) To the other paw of the same pair (*B* in fig. 1) was administered first 1 mg HSC and, 30 minutes later, 10 μg 48/80. Only 2 μg histamine was liberated from this paw. Five milligrams of HSC to a third paw completely blocked the action of 1 μg 48/80 (*C* in fig. 1). As shown in fig. 1, the addition of 5 mg HSC caused a transient reduction of the perfusion flow. Aside from this transient drop in the perfusion rate, HSC did not markedly influence the perfusion.

As reported by HÖGBERG et al. (1956), the two fore or hind paws from one and the same cat usually present fairly close agreement in regard to the amount of histamine released by 48/80. Table 1 contains the results obtained from 6 pairs of paws that received 10 μg 48/80. One paw in each pair was pretreated with HSC 30 minutes or more before the addition of 48/80. In all paws pretreated with HSC the release of histamine was lower than in the control paw; indeed, the liberation was reduced to insignificant amounts in some of the experiments. HSC also inhibited

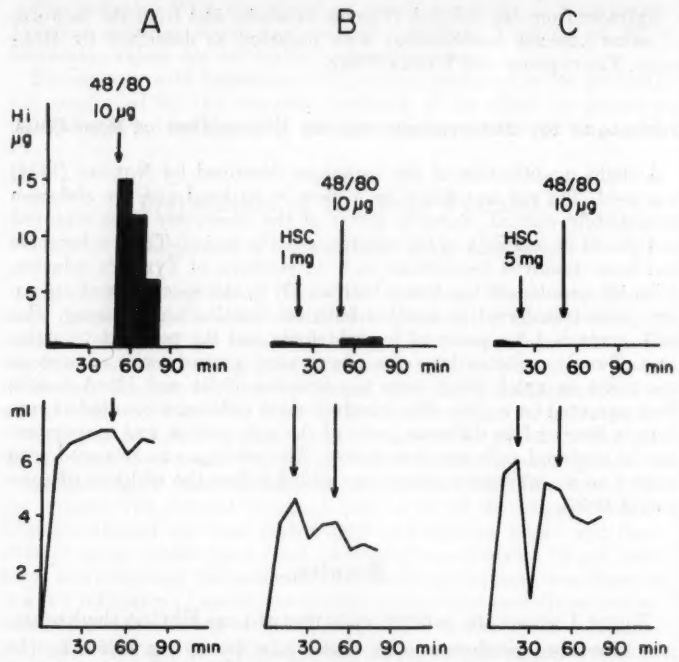

Fig. 1. Inhibitory effect of HSC on the histamine liberating action of 48/80 in perfused cat paw. Below: Perfusion rate.
A. Control paw: 10 µg 48/80 liberates 34 µg hi.
B. After 1 mg HSC, 10 µg 48/80 liberates 2 µg hi.
C. After 5 mg HSC, 10 µg 48/80 liberates no hi.

the action of the histamine liberators contained in *Ascaris lumbricoides* and *Cyanea capillata* (see table 1 and fig. 2).

In five of six experiments in which the histamine content of the paws was determined after the end of perfusion, the inhibition of histamine release was reflected by a lower histamine content in the control paws than in those treated with HSC.

In all experiments except one, HSC significantly reduced the production of edema (see table 1).

The duration of the inhibitory action of HSC was not determined exactly but was observed to be at least 60—90 minutes in spite of continuous perfusion of the paw with Tyrode's solution.

Experiments were conducted in order to investigate any possible interference of HSC with the histamine assay. However, in

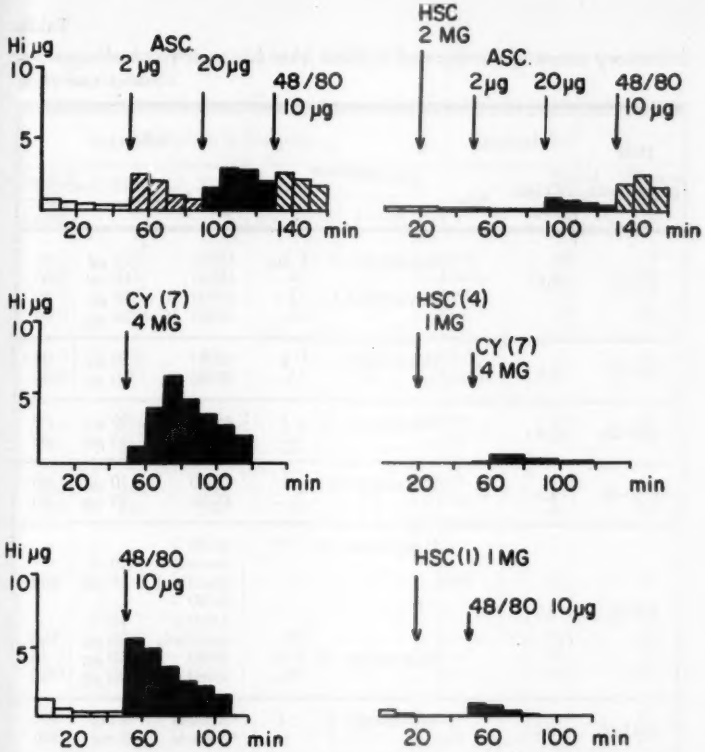

Fig. 2. Inhibitory action of HSC on the release of histamine produced in perfused cat paw by 48/80, and extracts from *Ascaris lumbricoides* and *Cyanea capillata*.

concentrations from 1 : 10,000 downward, HSC did not influence the histamine assay. Higher concentrations of HSC than 1 : 10,000 cannot reasonably be assumed to have occurred in the perfusion outflow after 30 minutes' continuous perfusion. In concentrations around 1 : 1,000, HSC increased the intestinal tone, with a tendency to spontaneous movements, but did not appreciably diminish the sensitivity of the gut to histamine.

The protective action of HSC against the disrupting action of 48/80 on mast cells is shown in fig. 3. In this experiment 48/80 in a concentration of 4 µg/ml caused disruption of 78 per cent of the mast cells. HSC in concentrations of 1 mg/ml and 100 µg/ml completely prevented the disruption. A concentration of 10 µg/ml

Table

Inhibitory action of a compound isolated from hip seeds on histamine re-
coides and from

Date of experiment	Animal		Inhibitor		Liberator		Liber.-time min.
	Weight kg	Sex			Amount		
4/5-55	3.8	—	Hip-extract 1	1 mg	48/80	10 μg	60
			—	—	48/80	10 μg	60
			Hip-extract 1	4.4	48/80	10 + 10 μg	90
			—	—	48/80	10 + 10 μg	90
6/9-55	3.0	♂	Hip-extract 1	4	48/80	10 μg	90
			—	—	48/80	10 μg	90
3/10-55	2.9	♀	Hip-extract 1a	1	48/80	10 μg	60
			—	—	48/80	10 μg	60
18/10-55	4.3	♂	Hip-extract C	1	48/80	10 μg	60
			—	—	48/80	10 μg	60
6/6-55	3.7	♂	Hip-extract 1a	9	48/80 + Ascaris (purified)	10 + 10 μg	100
			—	—	48/80 + Ascaris (purified)	10 + 10 μg	100
			Hip-extract 1a	9	48/80	10 μg	40
			—	—	48/80	10 μg	40
7/11-55	2.3	♀	Hip-extract C	1	*Ascaris	5 mg	60
			—	—	*Ascaris	5 mg	60
11/1-56	2.4	♀	Hip-extract 4	1	*Cyanea	4 mg	70
			—	—	*Cyanea	4 mg	70

* Crude preparations.

had a doubtful protective action, and 1 μg/ml was without any effect. Figure 3 also demonstrates that HSC was able to block the disruption of mast cells produced by an eelworm extract. In this experiment, 0.4 ml/ml of an *Ascaris* extract disrupted about 50 per cent of the cells. HSC in concentrations of 1 mg/ml and 100 μg/ml markedly inhibited the disruption. With a concentration of 10 μg/ml the inhibitory action was doubtful. One microgram per milliliter had no effect. HSC also inhibited the disrupting action of extracts from *Cyanea capillata* (not shown in fig. 3).

1.

lease in the cat paw produced by 48/80 and by extracts from Ascaris lumbri- Cyanea capillata.

Histamine in the paw					Paw		
Released μg	Remaining μg	Total μg	Liberated %	Released μg/g tissue		Initial weight g	Increase of weight %
2	55	57	4	0.05	hind	43	5
23	34	57	40	0.5	hind	45	18
1	67	68	1	0.03	fore	31	16
16	38	54	30	0.7	fore	29	28
0	183	183	0	0	hind	41	10
22	187	209	11	0.5	hind	41	37
16	173	189	8	0.6	fore	28	25
42	113	155	27	1.5	fore	29	48
26	259	285	9	0.6	fore	41	20
46	193	239	19	1.1	fore	40	13
4	—	—	—	—	hind	51	10
58	236	294	20	1.1	hind	52	29
0	210	210	0	0	fore	35	29
25	132	157	16	0.7	fore	35	100
8	—	—	—	0.3	fore	23	10
16	—	—	—	0.7	fore	22	18
3	—	—	—	0.09	hind	36	22
24	—	—	—	0.6	hind	37	41

Preliminary analysis of the HSC preparations suggested that the inhibitor was pectic acid with some of the carboxyl groups esterified (about 50 per cent). Further characterization of the inhibitor is in progress.

Discussion.

The present observations of the inhibitory action of HSC upon the histamine liberation and the disruption of mast cells produced by 48/80 and other histamine liberating agents raise several questions.

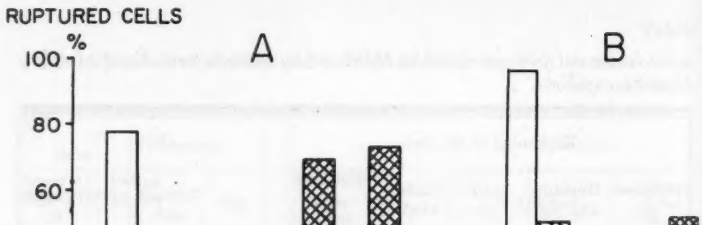

Fig. 3. Inhibitory action of HSC on the disruption of mast cells produced by 48/80 and an extract from *Ascaris lumbricoides*.

A.	% Ruptured cells
1. NaCl	3
2. 48/80 4 μg	78
3. NaCl + HSC(4) 1 mg	4
4. 48/80 4 μg + HSC(4) 1 mg.	2
5. NaCl + HSC(4) 100 μg	2
6. 48/80 4 μg + HSC(4) 100 μg	6
7. NaCl + HSC(4) 10 μg	8
8. 48/80 4 μg + HSC(4) 10 μg	69
9. NaCl + HSC(4) 1 μg	4
10. 48/80 4 μg + HSC(4) 1 μg.	73

B.	% Ruptured cells
1. NaCl	5
2. 48/80 5 μg	96
3. Ascaris (12) 0.4 ml	50
4. Ascaris (12) 0.4 ml + HSC(4) 1 mg	8
5. Ascaris (12) 0.4 ml + HSC(4) 100 μg	12
6. Ascaris (12) 0.4 ml + HSC(4) 10 μg	39
7. Ascaris (12) 0.4 ml + HSC(4) 1 μg	51

To which substance or substances in the seed extracts can the inhibitory action be ascribed?

Since chemical analysis of the hip extracts showed them to consist almost exclusively of pectic substances, it is plausible to assume that the inhibitory action of HSC is confined to these polysaccharide derivatives.

Rocha e Silva and Grãna (1946) reported that glycogen administered intravenously in a dog was able partially or completely to block the shock produced by intravenous injection of *Ascaris* extract. The liberation of histamine and heparin from the liver was prevented. The authors ascribed the action of glycogen to the following mechanism. *Ascaris* extract was assumed to produce vasoconstriction in the liver, and agglutinates of leucocytes and thrombocytes lodged in the liver capillaries. These microthrombi

disintegrated and furnished an enzymatic factor, possibly a tryptase, which liberated histamine and heparin from the liver parenchyma, and shock occurred. Since intravenous administration of glycogen caused marked leucopenia and thrombocytopenia, the protective action of glycogen against shock was attributed to its effect on the blood elements. Removal of the leucocytes and thrombocytes from the circulating blood should prevent the formation of agglutinates and microthrombi in the liver capillaries, and hence the occurrence of shock.

In our experiments the paw was perfused with Tyrode's solution, and it must have been rinsed virtually free of blood before the HSC was added to the perfusion fluid. The inhibitory action of the extracts cannot, therefore, have been due to elimination of leucocytes and thrombocytes from the perfusion fluid. Since 48/80 has been shown to liberate histamine by causing rupture of mast cells, one explanation might be that HSC prevents the rupture of mast cells in the perfused paw. Such a hypothesis is supported by the observation that HSC is able to prevent or reduce the rupture of mast cells caused by 48/80 *in vitro*.

Hip seed compound blocked not only the histamine liberation and mast cell disruption produced by 48/80, but also the similar actions of extracts from jellyfish and eelworm. It is not yet possible to evaluate the significance of this observation, but it might indicate either that the three liberators are chemically closely related and have the same point of attack, or that they attack at different points in a series of reactions which terminate in the release of histamine. In this case the inhibitory factor would probably block a process common to the liberating action of the three substances.

The intimate mechanism of inhibition is obscure. It is known that polysaccharides and other polymer compounds are able to block enzymatic processes. One plausible hypothesis is, therefore, that the polysaccharides in the hip seed extracts produce inhibition by interfering with some enzymatic process involved in the histamine liberation caused by the liberators studied.

Since the HSC presumably consists of substances of high molecular weight, it seems most reasonable to assume that this inhibition takes place at the surface of the histamine retaining cells. As mentioned above, the inhibitory action of HSC on the histamine release in cat paw persists for at least 60—90 minutes notwithstanding continuous perfusion of the paw with Tyrode's

solution. One explanation of this observation might be that the HSC in some way adheres to the cell surfaces. However, another and perhaps simpler explanation could be that in spite of the perfusion with Tyrode's solution, enough HSC remains in the perfusion fluid to "neutralize" the histamine liberating agents. Investigations are in progress to elucidate the mode of action of the polysaccharides.

Summary.

Hip seed extracts prepared by repeated precipitation with alcohol or trichloracetic acid extraction and barium fractionation were observed to block:

(1) The histamine liberation produced in perfused cat's paw by 48/80 and by extracts from jellyfish (*Cyanea capillata*) and the eelworm of swine (*Ascaris lumbricoides*).

(2) The rupture of mast cells produced by the same three agents.

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Increase in Noradrenaline Excretion Following Activation of the Vasomotor System during Tilting in Adrenalectomized Patients.

By

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Received 12 October 1956.

Tilting of normal healthy subjects from the recumbent position to + 75° is accompanied by an increased urinary output of noradrenaline as previously reported by EULER, LUFT and SUNDIN (1955). This is assumed to be due partly to an activation of the vasomotor system and partly to increased secretion from the adrenal medulla since it is known that diminution or exclusion of the baroceptor impulse activity from the carotid sinus, as a result of carotid occlusion, is followed by an increased output of noradrenaline from the adrenal medulla in the cat (KAINDL and EULER 1951) in addition to increased vasomotor activity. Normally it is not possible to state which of these factors is the predominant cause of the increased noradrenaline excretion in man. After adrenalectomy, however, it has been shown that the patients excrete either no or very little adrenaline in comparison with normal subjects, but still excrete noradrenaline in apparently normal quantities (EULER, FRANKSSON and HELLSTRÖM 1954). Since the noradrenaline excretion in this case cannot be derived from the adrenal medulla it is concluded that it originates from the adrenergic nerve endings, and primarily from the vasomotor nerves which constitute the quantitatively most important part of the adrenergic system.

In order to study the question of whether the increase in noradrenaline excretion in urine, observed on tilting normal subjects head-up + 75° is due primarily to activation of the vasomotor

system or to the adrenal medulla, we have studied the catechol amine output in urine in adrenalectomized patients before and during tilting. The results would be expected to give an answer to the debated question as to whether a reflexly induced increased activity of the adrenergic vasomotor system gives rise to an increased release of noradrenaline into the circulating blood, reflected in a higher urinary output of the neurotransmitter.

Material and Methods.

The present series comprises 10 females suffering from mammary cancer with metastases in which bilateral adrenalectomy + ovariectomy had been carried out. Their ages ranged from 38 to 56 years. The patients were on a substitution therapy of 50—75 mg cortisone daily by mouth. At the time of investigation they all were in excellent condition and lived a practically normal life.

The present tests were made at various times after the operation, more than two years in four patients, one year in two patients and from two to six months in the remaining four. Urine was collected during one to five 24-hour periods before the tilting tests in 9 of the patients, and the catechol amine excretion measured. In order to compare the adrenaline and noradrenaline excretion during recumbent position and during tilting, urine was collected for 3 hours between 7 and 10 a.m. and then during 1 hour on the tilting table at an angle of + 75°. In a few experiments the patient remained on the tilting-table for a further hour until noon.

The urine was kept cold during the collection period and acidified to pH 2—3 with HCl. The catechol amines were adsorbed on aluminium oxide and eluted with sulphuric acid according to the method of Euler and Orwén (1955). Quantitative estimation of adrenaline and noradrenaline separately was made by the biological assay method on the cat's blood pressure and the chicken rectal caecum as described by Euler and Hellner (1951). All figures are given in terms of the hydrochlorides of the catechol amines and no correction is made for the loss of about 25 % during the preparation.

The excretion figures are given as μg per 24 hours or as mμg/min.

Results.

The results are given in Table I where all individual excretion figures are included.

From the Table it can be seen that the noradrenaline excretion figures were increased during tilting in all 10 cases, on an average to twice the value during recumbency. The mean difference, 17 mμg/min., was highly significant.

Table I.

Adrenaline and noradrenaline excretion in μg during 24-hour periods and during recumbent and head-up position + 75° in adrenalectomized patients in mμg/min.

Pat.	24-hour periods		I Recumbency		II Head-up position + 75°		Difference	
	Adr.	Noradr.	Adr.	Noradr.	Adr.	Noradr.	II Adr.	I Noradr.
V. V.	—	—	1.1	15	0.0	30	— 1.1	15
S. J.	0.57 0.49 (0.53)	15 17 (16)	0.57	13	1.5	17	0.93	4
J. S.	0.90 0.38 (0.64)	23 29 (26)	0.54	25	1.1	35	0.56	10
E. P.	0.30 0.0 1.04 1.25 (0.65)	11 14 15 20 (15)	0.27 0.0 (0.14)	13 27 (20)	1.1 0.0 (0.55)	53 56 (55)	0.41	35
A.-B. J....	0.0	43	0.0	28	2.5	55	2.5	27
M. L.	0.0 0.12 (0.06)	28 16 (22)	0.21	12	0.32 0.13 (0.23)	27 26 (27)	0.02	15
A. W.	1.1 1.2 (1.2)	13 19 (16)	1.0	16	0.36	35	— 0.64	19
E. B.	0.0 0.16 0.08 1.2 0.42 (0.37)	27 5.1 32 40 24 (26)	0.16	20	0.30	31	0.14	11
S. A.	0.31	18	0.15	15	0.0 0.06 (0.03)	33 25 (29)	— 0.12	14
F. M.	0.83 0.68 (0.75)	19 17 (18)	0.0	18	0.62	38	0.62	20
Mean ± S. E.	0.50	22	0.39 ± 0.13	18 ± 1.6	0.72 ± 0.25	35 ± 3.8	0.33 ± 0.28	17 ± 2.8

The adrenaline excretion was only slightly increased during tilting in 7 cases, the mean difference being statistically insignificant.

Discussion.

There can be little doubt that the increase in noradrenaline excretion observed during tilting is due to an increase in the vasomotor activity induced by this procedure and that, consequently, such activity is accompanied by an overflow of transmitter substance. This is of some interest to state since it has been doubted by some authors that the physiological activity of the adrenergic system can give rise to an overflow of noradrenaline into the circulating blood. This must be the case, however, under the present circumstances, since there is no evidence that other extra-adrenal sources of noradrenaline in adult organisms, such as chromaffin cell tissue, could account for the relatively high excretion figures.

Chromaffin cell activation as a cause of urinary noradrenaline during the prevailing conditions is unlikely also in view of the very small adrenaline excretion, which is in accord with previous observations of EULER, FRANKSSON and HELLSTRÖM (1954), and the insignificant change in adrenaline excretion during tilting.

The results also lend weight to the conclusion that the production of adrenal medullary hormones is in no way a prerequisite for the synthesis of noradrenaline in the adrenergic neurones, as has been held by some authors.

It is also interesting to note that the noradrenaline excretion figures in the adrenalectomized patients are not lower than those found in a group of healthy subjects during recumbency as well as in erect position. EULER, LUFT and SUNDIN (1955) found a mean noradrenaline excretion in recumbency of 12.3 ± 3.1 mμg/min. as compared with the present figure of 18 ± 1.6. The corresponding figures during tilting were 30.9 ± 4.1 in the normal persons as against 35 ± 3.8 in the adrenalectomized patients. While the difference between the excretion figures during tilting in the two groups is not statistically significant there is a clear tendency towards higher noradrenaline excretion figures in the adrenalectomized patients during recumbency than in normal subjects. It should also be mentioned that no signs of vasoregulatory insufficiency were noted in our patients.

Summary.

The noradrenaline excretion figures in adrenalectomized patients were significantly increased during tilting head-up + 75° as compared with the figures during recumbency, indicating that reflex activation of the vasomotor system causes an overflow of neurotransmitter into the circulating blood from where it can be partly recovered in the urine.

This work has been supported by a grant from the Knut and Alice Wallenberg Foundation which is gratefully acknowledged.

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Histamine Degradation in Tissue Extracts from the Cat.

By

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Until some years ago the only known pathway for the degradation of histamine in the body was the oxidative deamination described by BEST and MC HENRY (1930) and MC HENRY and GAVIN (1932). These authors gave the enzyme responsible for the reaction the name histaminase. ZELLER (1938) has presented evidence that the same enzyme acts on diamines such as cadaverine and has therefore proposed the name diamine oxidase.

In a series of publications SCHAYER and his collaborators (for references see KARJALA, TURNQUEST and SCHAYER 1956) have described the action in intact animals of another enzyme system that destroys histamine. According to SCHAYER, KENNEDY and SMILEY (1953) this "histamine-metabolizing enzyme II" plays the major rôle in the destruction of histamine in young cats. The "enzyme II" has no part in cadaverine metabolism (SCHAYER, SMILEY and KENNEDY 1954).

As several investigators (HOLTZ, HEISE and SPREYER 1938, HAEGER and KAHLSON 1951) have dealt with histamine inactivation in extracts of organs from the cat as caused by histaminase (diamine oxidase) it was considered to be of interest to study if "histamine-metabolizing enzyme II" takes part in the histamine degradation caused by tissue extracts from feline kidney and

intestinal mucosa. The qualities of the enzymatic activity in these extracts have been studied and compared with those of hog kidney diamine oxidase, which has been extensively studied by ZELLER and others (ZELLER 1951, TABOR 1954). The results indicate that the degradation of histamine in extracts of kidney and small intestinal mucosa of the cat is due to the action of diamine oxidase.

Materials and Methods.

Adult cats weighing 2—4 kg were used. Extracts of the kidney and small intestinal mucosa were obtained by mincing the tissue in a Waring blendor with 0.2 M sodium phosphate buffer pH 7.2 (2.5 ml of buffer for each gram of tissue) and centrifuging. Pig kidney extracts were obtained by gentle shaking of one part acetone powder, prepared from pig kidney cortex according to TABOR (1951), in ten parts of the mentioned buffer for 30 minutes. Both these enzyme preparations are referred to as tissue extracts in the following.

Catalase was prepared from hog blood as described by BONNICHSEN (1955).

Histamine and cadaverine were used as the dihydrochlorides. Aminoguanidine bicarbonate was kindly supplied by Fluka AG, Buchs, St. Gallen, Switzerland. 1-isopropyl-2-isonicotinylhydrazine ("Marsilid") and 1-isobutyl-2-isonicotinylhydrazine dihydrochloride (IBINH) were kindly supplied by Hoffman-La Roche AG, Basel, Switzerland.

Determinations of enzyme activity.

The oxygen consumption of the tissue extracts with histamine or cadaverine as substrates was determined manometrically by the Warburg technique. The incubations were undertaken at 37.0° C. Readings were made every 10 minutes for 1 to 3 hours. The K_{O_2} for the Warburg vessels varied from 0.75 to 1.20. The values for oxygen consumption were corrected for blank oxygen uptake. KOH was added to the centre well. Substrates, inhibitors and catalase were added from the side arms. The initial velocity of the oxygen consumption was derived from the curve of oxygen uptake as a function of time.

The loss of biological activity of histamine was followed under conditions similar to those prevailing in the manometric experiments. Histamine and tissue extract were mixed thoroughly, put in test tubes and shaken in a water bath at 37.0° C for periods up to 4 hours. At the time of mixing of histamine and extract, and at suitable time intervals, a measured volume of fluid was rapidly transferred into a known amount of Tyrode's solution, kept at 100° C. This arrested the enzyme activity. The samples were then, if necessary, diluted and assayed for histamine activity on guinea-pig ileum. It was first ascertained that the sample taken immediately after mixing contained the added amount of histamine. The other samples were then compared with this sample, which was used as a standard.

Results.

It was found that addition of histamine to the tissue extracts caused an oxygen uptake. A concomitant disappearance of biological activity was observed. The enzymatic oxygen consumption and histamine inactivation were studied over a wide range of substrate concentrations. The observations on substrate concentrations below 1×10^{-3} M were made with bioassay alone and required dilution of the tissue extract. A dilution per se did not change the specific activity of kidney extracts. In fresh extracts of small intestinal mucosa a dilution sometimes resulted in increased specific activity. This effect of dilution did not occur when the extracts had been stored for some time at 0° C.

The reaction rates for kidney extracts at various initial histamine concentrations as the per cent of the rate at an initial concentration of 2×10^{-3} M histamine are shown in fig. 1. Similar results were obtained for intestinal extracts. The curves have the same form as that given by ZELLER (1939) for a partly purified diamine oxidase. The MICHAELIS' constants were calculated to be about 4×10^{-5}.

Histamine and cadaverine were incubated with the tissue extracts separately and simultaneously. The ratio between cadaverine oxidation and histamine oxidation was between 2 and 3 in both pig and cat tissue extracts. In the presence of both amines the reaction rate was intermediate.

Total oxygen consumption. It is generally accepted that the mechanism for the degradation of histamine and diamines as cadaverine by diamine oxidase is

$$RCH_2NH_2 + H_2O + O_2 = RCHO + H_2O_2 + NH_3$$

In the present experiments the oxygen uptake decreased considerably or stopped entirely when 0.5 mole of oxygen per mole of available substrate had been consumed. Addition of new histamine caused an increase in oxygen consumption, indicating that the enzyme was still active. The total oxygen consumption in our experiments was thus only half of that predicted by the given formula. This might be due to the presence of catalase in amounts sufficient to decompose all peroxide formed (TABOR 1951).

To test this hypothesis manometrical and biological determinations were made on tissue extracts with addition of catalase. No differences were observed. In the manometrical experiments without catalase, subsequent addition of catalase did not release

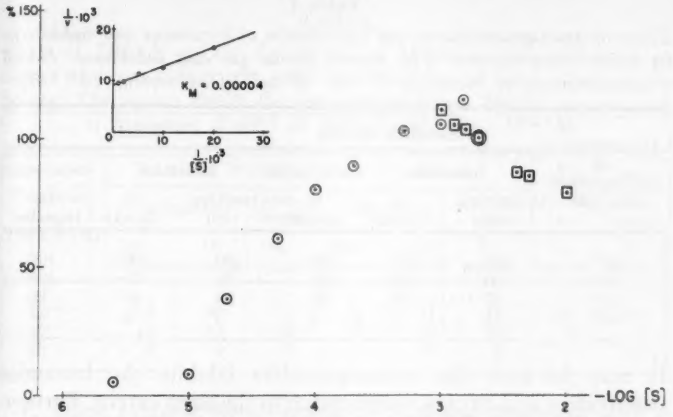

Fig. 1. Extract of cat kidney. Effect on reaction rate of varying the initial concentration of histamine. The reaction rates at various molar concentrations of histamine are given as per cent of the rate at a concentration of 2.10^{-3} M histamine.
○ activity determined by bioassay
□ » » from oxygen uptake.

any oxygen. Addition of peroxide on the other hand resulted in a sudden release of oxygen. The amount of peroxide formed was demonstrated by addition of ethyl alcohol to make a final concentration of 1 %. This increased the oxygen consumption with nearly 100 %. Under these conditions the decomposition of peroxide under oxygen liberation is replaced by coupled oxidation of the ethyl alcohol (KEILIN and HARTREE 1945, KAPELLER-ADLER 1949).

The results of the experiments in this section make it likely that the catalase activity in the extracts was sufficient to decompose all peroxide formed, which would liberate 0.5 mole of oxygen per mole peroxide.

Enzyme inhibitors. Aminoguanidine is a very powerful inhibitor of diamine oxidase in vitro (SCHULER 1952). According to SCHAYER et al. (1953) aminoguanidine does not affect the activity of "histamine metabolizing enzyme II". The second enzyme is, however, inhibited by "Marsilid" and IBINH (SCHAYER 1956).

The inhibitory effect of aminoguanidine on histamine inactivation and on oxygen consumption with histamine or cadaverine as a substrate was studied. Substrate and inhibitor were added simultaneously to the extract. The results are given in table 1.

Table 1.

Effect of aminoguanidine on the degradation of histamine and cadaverine by feline tissue extracts. The figures denote per cent inhibition. Initial concentrations of histamine $2 \cdot 10^{-3}$ M and of cadaverine 10^{-3} M.

Concentration of aminoguanidine (M)	Intestinal Mucosa			Kidney		
	histamine		cadaverine	histamine		cadaverine
	inactivation	O_2 uptake	O_2 uptake	inactivation	O_2 uptake	O_2 uptake
10^{-4}	100	100	100	100	100	100
10^{-5}	90	96	100	83	75	100
10^{-6}	37	35	32	33	45	90
10^{-7}	15	6	0	0	5	—

It may be seen that aminoguanidine inhibits the histamine inactivation and oxygen consumption to the same extent. Furthermore aminoguanidine suppressed the enzyme activity almost completely in a concentration of 10^{-4} M or 10^{-5} M.

The inhibitory effect of "Marsilid" on the histamine inactivation and on enzymatic oxygen consumption was studied in different types of experiments. No inhibition of either inactivation or oxygen consumption was observed until the tissue extract was pretreated at 37° C with "Marsilid" in 10^{-4} to 10^{-3} M concentrations for periods up to one hour. Under these conditions the degradation of cadaverine was depressed to the same extent as that of histamine. This was the case both with cat and pig tissue extracts. The inhibition by "Marsilid" increased with decreasing substrate concentrations.

The effect of IBINH on histamine inactivation and on enzymatic oxygen consumption are shown in table 2. Substrates and inhibitors were added simultaneously in the experiments represented in the table. If the IBINH was allowed to exert its effect on the enzyme before addition of substrate the inhibition was found to be more pronounced. Like "Marsilid" IBINH inhibited the oxidation of histamine and cadaverine as well as the biological inactivation of histamine in about the same degree. IBINH was, however, effective in lower concentrations than "Marsilid".

Discussion.

It would seem that diamine oxidase is present in our extracts from the feline kidney and intestinal mucosa. The evidence for this is:

Table 2.

Effect of IBINH on the degradation of histamine and cadaverine in extract of feline kidney and small intestinal mucosa and extract of pig kidney. The figures denote the per cent inhibition. Initial concentration of histamine $2 \cdot 10^{-3}$ M and of cadaverine 10^{-2} M.

Concentration of IBINH (M)	Intestinal Mucosa			Cat Kidney			Pig Kidney	
	histamine		cadaverine	histamine		cadaverine	histamine	cadaverine
	inactivation	O_2 uptake	O_2 uptake	inactivation	O_2 uptake	O_2 uptake	O_2 uptake	O_2 uptake
10^{-3}	54	40	71	88	71	85	100	100
10^{-4}	15	18	20	14	0	32	0	5
10^{-5}	0	0	0	5	0	0	—	—

1. These extracts were capable of oxidizing histamine with a concomitant inactivation of the amine.

2. The cat tissue enzyme was inhibited by histamine in concentrations slightly above that which gave a maximal reaction velocity.

3. The extracts oxidized cadaverine as well and the reaction velocity was higher with cadaverine than with histamine. In the presence of both amines the reaction rate was intermediate.

In these aspects the enzyme activity in our extracts has the characteristics, which ZELLER (1951) has given for diamine oxidase.

4. The total oxygen consumption found in the present experiments was half a mole of O_2 per mole of added substrate. This is in agreement with the over-all reaction for diamine oxidase activity in crude extracts containing catalase given by TABOR (1951).

$$RCH_2NH_2 + {}^1/_2\ O_2 = RCHO + NH_3.$$

Recent investigations (SCHAYER and KARJALA, 1956) show that "histamine-metabolizing enzyme II" is a composite of a methylating enzyme and oxidative enzymes. Histamine is first methylated to 1-methyl-4-aminoethyl-imidazole, which is pharmacologically inert (LEE and JONES 1949). The methylated histamine is then oxidized to methylimidazole acetic acid. The inhibitor IBINH blocks the oxidation of 1-methyl-4-aminoethyl-imidazole but does not interfere with the methylation.

The oxidation of histamine by "enzyme II" is not sensitive to aminoguanidine. The complete inhibition by aminoguanidine of

the histamine degradation in the present experiments therefore makes it unlikely that "enzyme II" is active under the prevailing conditions. The inhibitory effect of high concentrations of "Marsilid" and IBINH in our experiments might be interpreted as an action on "enzyme II". A more likely explanation is that these substances have a direct inhibitory action on diamine oxidase. This hypothesis is supported by the observation that they depressed histamine inactivation and histamine and cadaverine oxidation in the same degree.

Summary.

1. The degradation of added histamine by crude extracts of kidney and small intestinal mucosa from adult cats was studied by measuring the oxygen consumption manometrically and by observing the loss of biological activity.

2. It was found that these extracts contained a histamine degradating enzyme with the characteristics of diamine oxidase.

3. No conclusive evidence was found in these experiments for the action of a histamine inactivating enzyme other than diamine oxidase.

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The Effects of Cooling the Feet and Closing the Eyes on Standing Equilibrium. Different Patterns of Standing Equilibrium in Young Adult Men and Women.

By

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Received 23 October 1956.

Standing equilibrium is in no sense a static condition, but a dynamic phenomenon, movement upon a stationary base (HELLEBRANDT 1938). Postural muscle contractions, stretch reflexes, are very important in dynamic standing equilibrium. In stretch reflexes, afferent impulses are stimulated by stretching the muscle. It is also well known that sight is important in the maintenance of standing equilibrium (Romberg test), and it seems that the cutaneous sensibility of the soles participates in it too. HARRIS (1938) has shown that faradic stimulation of low threshold cutaneous fibers in the medial plantar nerve in dogs gives rise to an ipsilateral extensor response pattern, which must be considered a slow postural response. HEYD (1862, quoted by HARRIS) found that subjects standing in a cold salt solution or with the soles anesthetized by immersion in chloroform exhibit great difficulties in keeping their standing equilibrium. On the other hand, the vestibular apparatus seems to play no important part in the maintenance of standing equilibrium (WIGGERS 1949).

It has been found that *e. g.* old age (FRUCHT 1952, BOMAN and JALAVISTO 1953), experimental insomnia (LEE and KLEITMAN 1923) and alcohol intoxication (GOLDBERG 1943) increase swaying in standing posture. In an extensive series of cases FRUCHT observed that swaying in women aged from 15 to 30 years is noticeably larger than that in men of the same age.

Material and Methods.

All the subjects were students at the Institute of the Physical Education, University of Helsinki, and were healthy vigorous persons. They were from 19 to 25 years of age.

Swaying was registered by an optic method, modified from GOLDBERG's method (1943) by BOMAN and JALAVISTO (1953). The movements of an illuminated point on the head of the subject was photographed with the camera above the subject. The picture formed by the swaying spot of light on the film was projected on to a scale, and the extents of the antero-posterior and transverse swings were measured. In all the experiments the time of registration was 1 min. and the subjects were standing barefoot with their feet together in a dimly lit room.

The effects of cooling the feet and closing the eyes were investigated as follows: the first registration of the sway was made when the subjects were standing on the floor with their eyes open, the second when they were standing with their eyes closed, the third when they were standing in ice water with their eyes open, and the fourth when they were standing in ice water with their eyes closed. The time between the first and the second, and between the third and the fourth registration was 1—2 min., during which time the subjects remained standing. The time between the second and the third registration was about 5 min., and during this interval the subjects were sitting and holding their feet in ice water. The object of the ice water experiments was to study the effect of the numbness of the feet on standing equilibrium. Complete anesthetization was, of course, not achieved by this method, but cutaneous cooling of this degree clearly decreases the sensibility of the skin, as MACKWORTH (1953) has shown in his experiments on the effect of cooling the fingers on two-point tactile discrimination.

In addition to these experiments two control experiments were carried out. To find out the effect of standing as such, the sway was registered four times at intervals of about 2 min., the subjects remaining standing on the floor with their eyes open. The effect of an uncomfortable sensation of cold as such was studied by registering the swing first when the subject was standing on the floor with his eyes open and then when he was holding one hand in ice water, the conditions being otherwise the same.

In all the experiments, the individual differences in sway between the different experimental situations were calculated and treated according to the conventional statistical methods.

Results.

Swaying when standing on the floor with the eyes open (Table 1). In women, the mean of the transverse swing was 40.5 ± 1.7 mm, and that of the antero-posterior swing 46.7 ± 1.6 mm. The corresponding values in men were 32.4 ± 1.3 mm and 38.5 ± 1.5 mm. Swaying was significantly wider in women than in men, the

Table 1.

The Amplitude of Swaying When Standing on the Floor with the Eyes Open.

	Women		*Men*	
	Transverse Swing (mm)	Antero-Posterior Swing (mm)	Transverse Swing (mm)	Antero-Posterior Swing (mm)
Number of Subjects: 45				
Range	18—83	28—73	13—80	20—86
Mean	40.5	46.7	32.4	38.5
Standard Error of the Mean	1.7	1.6	1.3	1.5
Standard Deviation	11.2	10.7	8.7	10.4

transverse swing in women being, on an average, 8.1 ± 1.7 mm and the antero-posterior swing 8.2 ± 1.8 mm greater (Table 2). The antero-posterior swing proved to be 6.3 ± 2.1 mm wider than the transverse swing in women and 6.0 ± 1.4 mm wider in men (Table 3).

Table 2.

Difference, the Amplitude of Swaying in Women Minus That in Men.

	Transverse Swing (mm)	Antero-Posterior Swing (mm)
Number of Subjects: 45		
Mean of Differences	8.1	8.2
Standard Error of the Mean	1.7	1.8
t	4.7	4.7
P	0.001	0.001

The effect of standing as such on the amplitude of swaying (Table 4). The control experiment showed that, on an average, no changes occurred in the magnitude of swaying during continuous standing for 11—12 min.

The effect of cooling the hand on the amplitude of swaying (Table 5). These experiments showed that the uncomfortable sensation of cold, produced by holding one hand in ice water, did not increase the swaying, on the contrary, the swinging tended to decrease. The subjects felt that holding a hand in ice water was more uncomfortable than standing in ice water.

Table 3.

Difference, Antero-Posterior Swing Minus Transverse Swing.

	Women	Men
Number of Subjects: 45		
Mean of Differences (mm)	6.3	6.0
Standard Error of the Mean	2.1	1.4
t	3.0	4.3
P	0.01	0.001

The effect of standing in ice water on the amplitude of swaying (Table 6). In men, standing in ice water did not, on an average, change the magnitude of swaying. But in women standing in ice water produced a significantly wider sway than standing on the floor. When they were standing with their eyes open in ice water, the swaying was in transverse direction 26.1 ± 4.5 mm and in antero-posterior direction 24.4 ± 3.7 mm greater than when they were standing on the floor. When their eyes were closed the corresponding values were 37.0 ± 4.8 mm and 26.4 ± 5.4 mm.

The effect of closing the eyes on the amplitude of swaying (Table 6). In men swaying was not influenced by closing the eyes, but in women shutting out visual impulses clearly increased the swaying. In the latter it widened the transverse swing by 8.6 ± 3.3 mm and the antero-posterior swing by 7.1 ± 3.1 mm when they were standing on the floor, and by 18.3 ± 4.0 mm and 9.4 ± 5.6 mm respectively when they were standing in ice water. Standing with closed eyes, however, increased the swing less than standing in ice water.

Discussion.

In this investigation, carried out on young, healthy subjects, two different mechanisms in the maintenance of standing equilibrium were found to exist, one being typical of women and the other of men. In normal conditions, while the subjects were standing on the floor with their eyes open, swaying both in the transverse and in the antero-posterior direction proved to be considerably wider in women than in men. In addition, standing in ice water and shutting the eyes clearly increased swaying in women

Table 4.

The Effect of Standing as Such on the Amplitude of Swaying.

	Number of Subjects	Range	Mean	Standard Error of the Mean	t	P
Women, Transverse Swing						
Difference, the Second Registration Minus the First Registration (mm)	20	— 16 to 35	— 0.75	3.2	0.2	..
Difference, the Third Registration Minus the Second Registration (mm)	20	— 32 to 10	— 5.0	2.8	1.8	..
Difference, the Fourth Registration Minus the Third Registration (mm)	20	— 15 to 32	2.2	2.5	0.9	..
Women, Antero-Posterior Swing						
Difference, the Second Registration Minus the First Registration (mm)	20	— 28 to 22	— 0.35	2.8	0.1	..
Difference, the Third Registration Minus the Second Registration (mm)	20	— 37 to 38	— 0.15	4.4	0.0	..
Difference, the Fourth Registration Minus the Third Registration (mm)	20	— 41 to 24	— 0.20	3.7	0.1	..
Men, Transverse Swing						
Difference, the Second Registration Minus the First Registration (mm)	20	— 12 to 21	1.0	2.0	0.5	..
Difference, the Third Registration Minus the Second Registration (mm)	20	— 16 to 13	— 0.55	1.7	0.3	..
Difference, the Fourth Registration Minus the Third Registration (mm)	20	— 16 to 23	— 0.15	2.3	0.7	..
Men, Antero-Posterior Swing						
Difference, the Second Registration Minus the First Registration (mm)	20	— 15 to 20	— 2.3	1.9	1.2	..
Difference, the Third Registration Minus the Second Registration (mm)	20	— 18 to 30	3.0	2.8	1.1	..
Difference, the Fourth Registration Minus the Third Registration (mm)	20	— 30 to 15	0.05	2.1	0.02	..

Table 5.

The Effect of Cooling the Hand on the Amplitude of Swaying.

	Difference, the Amplitude With One Hand in Ice Water Minus the Amplitude without It (mm)	
	In Transverse Direction	In Antero-Posterior Direction
Women		
Number of Subjects: 25		
Range	— 28 to 36	— 23 to 11
Mean	— 4.3	— 3.6
Standard Error of the Mean	3.2	1.6
t	1.3	2.2
P	..	0.05
Men		
Number of Subjects: 20		
Range	— 23 to 5	— 36 to 12
Mean	— 8.8	— 5.1
Standard Error of the Mean	1.4	2.7
t	6.3	1.9
P	0.001	..

but not in men. In these conditions the change in the amplitude of swaying cannot be caused by standing as such, nor by the uncomfortable sensation of cold as such, as was found in the control experiments. These results show, not only that swaying in women in standing posture is wider than in men, but also that the control of standing equilibrium is in them different from that in men. In women, sight and the cutaneous sensibility of the soles are, by the side of other postural reflexes, of importance, but not in men, in whom other postural reflexes, obviously myotatic stretch reflexes, dominate over those stimulated by the soles and the sight.

Previously FRUCHT (1952) has found that at the age from about 15 (the youngest subjects in his series) to 30 years swaying in women is wider than in men. LIEBERT (1941) observed that his subjects fell into two groups, the one of them showing 2—3 times as wide swaying as the other. In his paper the sex of the subjects has not been taken into consideration. In the present study no clear distribution into two groups could be seen among the subjects of one sex, neither in the extent of swaying nor in the effects of standing in ice water or with closed eyes. In spite of this, it may be worth mentioning that in the extent of swaying in

Table 6.

The Effect of Standing in Ice Water and Closing the Eyes on the Amplitude of Swaying.

In the table I = swaying when standing on the floor with the eyes open
II = swaying when standing on the floor with the eyes closed
III = swaying when standing in ice water with the eyes open
IV = swaying when standing in ice water with the eyes closed

	Difference in Transverse Direction (mm)						Difference in Antero-Posterior Direction (mm)					
	Number of Subjects	Range	Mean	Standard Error of the Mean	t	P	Number of Subjects	Range	Mean	Standard Error of the Mean	t	P
Women												
II Minus I	26	— 25 to 40	8.6	3.3	2.6	0.02	25	— 15 to 55	7.1	3.1	2.3	0.05
III Minus II	26	— 35 to 87	18.1	5.5	3.3	0.01	24	— 25 to 58	18.2	4.9	3.7	0.01
IV Minus III	25	— 21 to 60	18.3	4.0	4.6	0.001	22	— 44 to 52	9.4	5.6	1.7	..
III Minus I	27	— 5 to 95	26.1	4.5	5.8	0.001	25	— 27 to 56	24.4	3.7	6.6	0.001
IV Minus II	24	3 to 111	37.0	4.8	7.7	0.001	22	— 24 to 72	26.4	5.4	4.9	0.001
Men												
II Minus I	20	— 21 to 22	0.6	2.5	0.24	..	20	— 36 to 32	2.0	3.8	0.53	..
III Minus II	20	— 18 to 41	2.2	3.3	0.67	..	20	— 22 to 21	1.9	2.9	0.66	..
IV Minus III	20	— 43 to 37	4.4	4.4	1.00	..	19	— 13 to 35	6.2	3.1	2.0	..
III Minus I	20	— 15 to 39	2.8	3.1	0.90	..	20	— 19 to 22	0.05	2.4	0.02	..
IV Minus II	19	— 20 to 52	7.0	3.7	1.90	..	19	— 15 to 41	4.8	3.4	1.41	..

women there seemed to be a tendency to an unsymmetrical two-peaked distribution, where the higher peak corresponded to a swing of 31—35 mm in transverse direction and 41—45 mm in anterior-posterior direction, and the lower peak to the swing of 71—75 mm in both the transverse and antero-posterior directions. However, the material is too small for information of this kind, and the results have been treated as if the distribution were normal.

It has been found that the weight perception is based on the double peripheral sensory basis, the peripheral mechanisms being partly tactile, due to the pressure on the skin and the other tissues, and partly proprioceptive, *i. e.*, due to the muscular tension developed (WANGEL, ELMGREN, v. BAGH and RENQVIST 1932). The share of these two components varies with external conditions. In their experiments with moderate weights, BOMAN and JALAVISTO (1954) found that in most people the perception is based diffusely on both peripheral mechanisms, but in the very old age almost solely on the tactile component. These, and other similar experiments (JALAVISTO 1935, REENPÄÄ and BOMAN 1954) show that the concept of specific receptors of the classical physiology is not correct. The theory of perception of REENPÄÄ (1953) is based partly on experiments of this kind. The results of the present investigation show that also in reflexes, at least in the postural reflexes which maintain the standing posture, the afferent part is not based on one peripheral sensory mechanism alone, but on many mechanisms, which may vary, at least according to the sex. The old mechanistic theory of reflexes has previously been proved to be incorrect, and the reflexes have been found holistic in their behavior, but mostly in experiments bearing on their efferent part (cf. v. WEIZSÄCKER 1927, BETHE 1931).

Is the different behavior of men and women in the maintenance of standing equilibrium only one of the sex differences, or can we find correlations to some other phenomena? On an average, the male subjects are taller than the female, but no correlation has been found between the height of subjects and amplitude of swaying (LIEBERT 1941, FRUCHT 1952, BOMAN and JALAVISTO 1953). Womens' and mens' gymnastics may differ, but training has been shown to have no effect on standing steadiness (LIEBERT 1941). Psychic attitude influences the amplitude of swaying, HELLEBRANDT (1938) and FRUCHT (1952) found, both in women.

that if the motivation to stand fixed was strong, swaying was really smaller than in other conditions. The great influence of personal factors appeared also in GOLDBERG's alcohol experiments (1943). On the basis of the present experiments it is impossible to determine the differences in the attitudes of men and women. If the difference observed in the maintenance of standing equilibrium were based on the divergent psychic attitudes, the attitude would not only affect the magnitude of swaying but also the mechanisms used in the maintenance of standing equilibrium. The most probable explanation seems to be the differences in muscular strength between the sexes. At every age muscular strength is considerably smaller in women than in men (REIJS 1921). The amplitude of swaying increases (FRUCHT 1952, BOMAN and JALAVISTO 1953), but muscular strength decreases (REIJS 1921, UFLAND 1933) with age. LEE and KLEITMAN (1923), who showed that during experimental insomnia swaying in standing posture increases, found also that during the experiment "a tendency toward muscular relaxation became evident and it was increasingly more difficult to keep the musculature tonus". According to them, the unsteadiness was possibly due to concomitant muscular fatigue. Many observations on standing steadiness can be explained by the following hypothesis: If muscular strength is great, muscular myotatic stretch reflexes are used almost exclusively in the maintenance of standing equilibrium, and in this case the amplitude of swaying is small; if muscular strength is not great, other afferent parts, the soles and sight play a more important role in postural mechanisms, and in this case the amplitude of swaying is wide.

Summary.

A clear sex difference in behavior in standing equilibrium has been observed. Swaying in young women is significantly wider than that in young men, and the numbness of the soles due to standing in ice water and closing the eyes noticeably increases swaying in women, but not in men. This difference between the sexes and some other observations on the maintenance of standing equilibrium can be explained by the following hypothesis: If muscular strength is great, muscular myotatic stretch reflexes are used almost exclusively in the maintenance of standing equilibrium, and in this case the amplitude of swaying is small. If muscular strength is not great, other afferent parts, the soles and sight

participate more in postural mechanisms, and then the amplitude of swaying is wide.

The statistical analysis has been made by Statistical Section, Institute of Occupational Health, Helsinki (Chief: J. KIHLBERG M. A.).

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Some Characteristics of Goldthioglucose Obesity in the Mouse.

By

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The pioneering work by HETHERINGTON and RANSON (1940), by BROBECK, TEPPERMAN and LONG (1943) and later by ANAND and BROBECK (1951) established, by the placement of lesions, the importance of the hypothalamus for the normal regulation of food intake. In addition, stimulation of the lateral parts of the hypothalamus was shown to result in a pronounced hyperphagia (BRÜGGER 1943, and LARSSON 1954).

When studying the problem of the regulation of food intake and the general characteristics of obesity it is of importance not only to obtain figures of the total food consumption, but also of eating behaviour continuously recorded over an extended period of time. BROBECK (1955) found it necessary to comment that "Nearly all of the published studies of the regulation of food intake neglect the fact that the total amount of food eaten is always the product of two factors, the number of meals multiplied by the intake of the average meal".

The present study deals with some behavioural characteristics in mice made obese by a single injection of goldthioglucose.

Methods.

Male albino mice were used for the experiments. They were kept on a standard diet (mouse crackers, KONSUM) and fed *ad libitum*. The weights of the animals were as a rule checked once a week.

The mice were maintained singly or in groups at a temperature of 24° C. When the mice were kept in individual cages the water consumption of the animals was recorded daily.

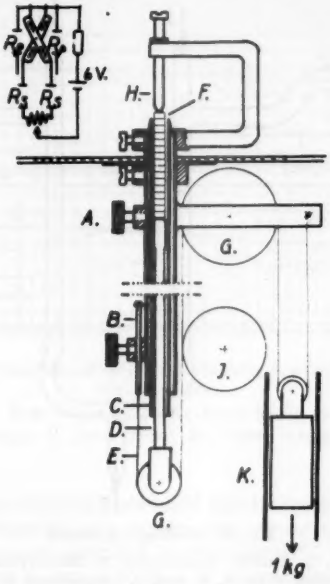

Fig. 1. "*Mouse feeder*".

A. Adjusting screw; *B.* Zero equaliser; *C.* Tablet container; *D.* Piston pushing the tablets upwards; *E.* Wire transferring the movements of piston by the wheels *G* to the potentiometer *J*; *F.* Tablets coming up against the stop *H*; *K.* Weight transferring pressure against the piston.

To produce the obesity, goldthioglucose as a sesam oil suspension ("Solganol B oleosum", SCHERING) was used. The injections were always made intraperitoneally in amounts corresponding roughly to the half lethal dose (1 mg per gram body weight). The most effective doses to produce the obesity have earlier been determined and studied by BRECHER and WAXLER (1943) and by DRACHMAN and TEPPERMAN (1954).

For the experiments only obese animals with a fully developed obesity were used. That is to say that they had passed the period of rapid weight gain. No special weight limit was set to determine which animals were to be regarded as obese because of reasons given under results and in the discussion.

In order to study the food intake and meal frequency as well as corresponding features for water intake, an apparatus to continuously record these parameters was designed. The recorder was a six colour "Siemens Fallbügelschreiber", permitting simultaneous recording of six different curves. This apparatus has a parallel coil movement, specially designed for recording from resistive transducers. Series

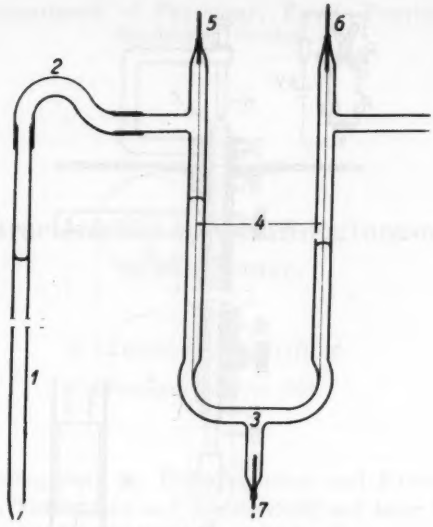

Fig. 2. *Drinking apparatus.*

1. Water container; 2. Rubber connection; 3. Mercuryfilled Y-tube of glass; 4. Platinum wire; 5., 6. and 7. connections to recorder.

connecting the coils transforms it to a very sensitive recording microammeter.

The light of the animal room was recorded through a selenium photocell and was usually kept on from 9 AM to 5 PM. The room temperature was measured by a resistance thermometer, giving a full scale deflection of only 2.9° C on the most sensitive range. The room temperature was kept constant at 24° C within $\pm$ 0.1° C by a thermostate and a fan circulating the air.

The food intake was measured by a special "feeder" (Fig. 1) and also continuously recorded. The "feeder" was loaded with a column of specially prepared tablets made out of the same standard diet as mentioned above, but also containing adhesive material (standard food 75 weight %, sacch. lact. 15 weight % and glucose lq. 10 weight %) in order to make the preparation of the tablets possible. In the "feeder" the column of tablets was pushed up against a stop by a piston, the movements of which were transferred to a 25 Ω, ten turn "Helipot". The resistance variations could then be transferred to the Siemens' recorder.

In one series of experiments the standard diet was diluted with kaolin as calorifically inert material, and tablets were made out of this mixture. The composition of this type of tablets was: standard diet 41.5 weight %, kaolin 41.5 weight %, sacch. lact. 8 weight %, glucose lq. 8 weight % and Mg-stear. 1 weight %.

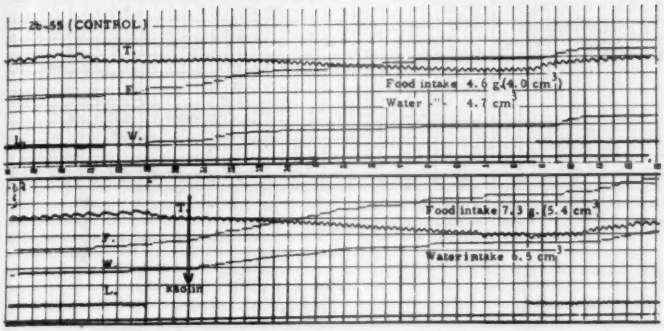

Fig. 3 A. Continuous recording of eating and drinking in a normal mouse receiving standard diet.

Fig. 3 B. Recording from the same mouse when kaolin is added to the diet. *T.* Temperature; *F.* Food intake; *W.* Water intake; *L.* Light.

The water intake of the animals could also be continuously recorded. The water was dispensed in a pipette, the upper end of which was connected to a pressure operated "potentiometer". The moving arm of the "potentiometer" was a column of mercury within which there was a platinum wire with a diameter of 0.03 mm, serving as the resistance of the "potentiometer" (Fig. 2). "Spontaneous" activity was measured by rotating drums — one turn on the drum corresponding to 50 cm run.

Results.

The results obtained with groups of mice which had reached a weight plateau are illustrated in the curves of Figs. 3 A and 4 A. The control mice ate more frequently than did the obese ones, whilst, on the other hand, the obese animals so far studied, consumed more food each time compared to their controls. The frequency of meals taken seemed by and large to be inversely correlated with the weight of the animals. Thus it was observed that the meal frequency in animals weighing 60—70 grams was about 9—15 meals in 24 hours. On the other hand the meal frequency in control animals weighing 20—25 grams varied between 35—45 times in 24 hours. In heavier controls weighing between 30—40 grams the corresponding meal frequency was around 20—25.

Conversely, the amount of food eaten at each meal showed a direct correlation with the weight of the animals.

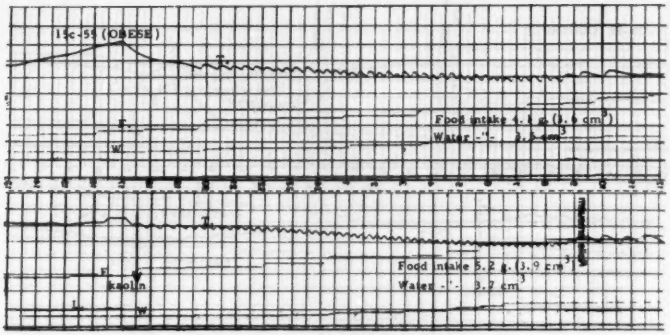

Fig. 4 A. Continuous recording of eating and drinking in an obese animal receiving standard diet.

Fig. 4 B. Recording from the same mouse when kaolin is added to the diet. *T.* Temperature; *F.* Food intake; *W.* Water intake; *L.* Light.

Preliminary experiments on young animals in a phase of rapid weight gain showed them to have a very high meal frequency. The same was true in mice of any age which had recently received a goldthioglucose injection and in which a phase of weight gain had thus been initiated.

No analyses have so far been made to see whether obese mice have a different 24 hours eating rhythm than the controls.

When kaolin was added to the standard food of the animals it was observed that the adult control mice maintained their weight satisfactorily. The animals compensated for dietary dilution either by increasing the frequency of meals, or by increasing the amount eaten each time. In some instances there was a combination of both these ways (Fig. 3 B). On the other hand the obese mice showed weight reduction when given kaolin and had little ability to compensate for the dietary dilution (Fig. 4 B). Growing young animals and newly injected ones, both groups being comprised of mice that were gaining weight fairly rapidly, usually plateaued at the weight, where kaolin was first incorporated in the diet.

In the "spontaneous" activity experiments it was found that the obese animals had a reduced initial activity (Fig. 5). However, if they were forced to run, which exercise as a rule resulted in a reduced weight, and after this were placed in the activity cages, their "spontaneous" activity was hightened. As a conse-

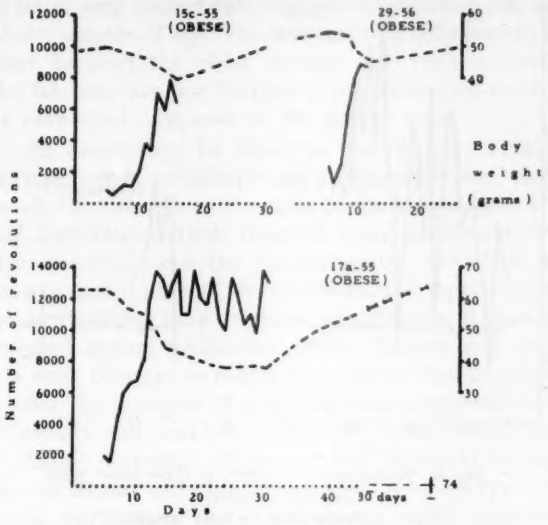

Fig. 5. "Spontaneous" activity in three obese mice.
Solid line — activity
Broken line — body weight

quence more weight was lost. One got the impression that the more weight they lost the more they ran until a body weight was reached at which the animals had the ability to keep caloric intake and energy expenditure equal. The control mice, often initially loosing a couple of grams, were rapidly able to equalise food intake and energy expenditure, thus maintaining a fairly constant weight (Fig. 6).

In the cases where water intake was recorded it was observed that drinking had a tendency to follow eating (Figs. 3 A and 4 A). In very few cases water intake of measurable amounts occurred without previous food intake. The water intake of the animals receiving food diluted with kaolin showed the same general characteristics as when a full diet was given (Figs. 3 B and 4 B), thus there were no relation between drinking and caloric density of the food but the volume eaten.

Discussion.

In the literature there are very few studies dealing with continuous recording of food and water intake. RICHTER (1927) demon-

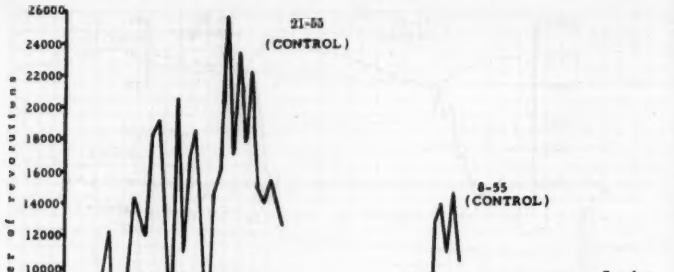

Fig. 6. "Spontaneous" activity in three obese mice.
Solid line — activity
Broken line — body weight

strated some general characteristics of eating and drinking behaviour in normal rats. Thus, he showed that eating and drinking is preceded by a gradually increased spontaneous activity of the animals. ANLIKER and MAYER (1956) used the "Skinner box" type of apparatus to get information on the eating behaviour in mice with different types of obesity. They pointed out the similarities between the eating behaviour of animals with hypothalamic obesity caused by electrolytic lesions and the type of obesity caused by injection of goldthioglucose. Their experiments showed that the mice with goldthioglucose obesity in contrast to the normal animals did not have the same cyclic variations in food consumption, and ate more evenly over the 24 hour period. The normal mice had evident 24 hour cyclical changes in the rate of feeding. In our studies the same eating characteristics were observed in the control animals. On the contrary, in the obese mice we have not obtained the same feeding curves as ANLIKER and MAYER (1956). In their work, however, no information was given whether the animals they investigated were rapidly gaining weight or if they had reached a weight plateau.

In the present investigation attention has been directed, mainly to the state of fully developed obesity in which the fat mice

showed no or very little weight increase. In addition our animals were over 9 months of age. The most striking difference in eating behaviour between the obese animals and their controls was that the fat mice ate less frequently but consumed much more food at each meal compared to the normal ones.

Since the observation by BRECHER and WAXLER (1949) that a single injection of goldthioglucose produced obesity, there has been much discussion as to the cause of the weight gain. DRACHMAN and TEPPERMAN (1954) reported many similarities between this type of obesity and the one caused by electrolytic lesions in the ventromedial parts of the hypothalamus, especially studied by BROBECK (1955). In a series of experiments MAYER (1955) also observed several similarities. OWEN, PARSON and CRISPELL (1953) in spite of negative results in trying to find hypothalamic lesions after the injection of goldthioglucose suggested that this type of obesity still could be caused by lesions in the "feeding center". Lately MARSHALL, BARRNETT and MAYER (1955) demonstrated such lesions and also showed that these always involved the medial portions of the hypothalamus which according to BROBECK (1955) involves the "satiety center". Such lesions were also observed in animals used in our experiments.[1] FORSSBERG and LARSSON (1954 and 1955) studied some metabolic processes in the brains of normal rats during hunger and satiety and found that the "feeding center", compared to other parts of the brain had an increased metabolic activity during hunger, especially in the adenosinetriphosphate and creatinephosphate fractions. Furthermore, there also seemed to be an increased blood supply to this part of the brain during hunger as compared to satiety. There is thus evidence for the existence of structures in the "feeding center" that are especially active during hunger and also with a selective sensitivity to goldthioglucose (LARSSON 1956). In the obesity caused by this compound the "satiety" part of the "feeding center" then is injuried and the animals no longer are able to regulate their food intake centrally by means of structures sensitive to variations in circulating metabolites. Therefore, peripheral regulators such as gastric motility and state of distension of the stomach might have more pronounced influence on the regulation of food intake in this type of obesity. It is suggested that the differences in eating behaviour between the

[1] The autopsies and the microscopical examinations were made by Prof. S. RUBARTH, Dept. of Pathology, Kungl. Veterinärhögskolan.

fat and normal animals depend on impairment of the "satiety center" *per se*. However, there is no reason to believe that the activity of the presumably unaffected lateral hypothalamus will be increased. As shown by BROBECK, LARSSON and REYES (1956) the medial and lateral parts of the "feeding center" are able to change their activity independently of each other. The drive to eat has a multiple origin (BROBECK 1955 and MAYER 1955) and the uptake of food is the result of the integrative action of different factors acting upon the "feeding center". In the case of the goldthioglucose obesity where structures in the "satiety center" with special chemo-sensitivity are damaged, the animals will be more dependent on "peripheral regulators" such as the gastrointestinal tract for the inhibition of food intake.

Then, in the fat animals the hunger contractions, or rather the emptying of the stomach to a certain degree, which takes away the inhibitory impulses arriving from the stomach during distension, would have an enhanced importance compared to the control animals. As shown by PAINTAL (1953) these inhibitory impulses are mediated via vagal afferents. Electrical stimulation in the region of the vagal nuclei (LARSSON 1954), and in the rostral mesencephalon and the posterior hypothalamus (RUCH, MAIRE and PATTON 1956) is capable of eliciting hyperphagia. There is thus evidence for a nervous connection between the stomach and the "feeding center". The inability of the obese mice to compensate for dietary dilution with kaolin with more frequent meals or to eat more each time might also be taken as support for the idea that in this type of obesity the importance of the gastrointestinal tract for the regulation of food intake is greater. The control mice seemed to compensate for the dilution with kaolin in different ways. In some cases the meal frequency increased; in others the amount of food eaten each time was larger, and finally in many instances there was a combination of both. Support of a slightly different nature for the conception of the increased importance of the gastrointestinal tract in obese animals, is the fact that where such obesity has been caused by electrolytic lesions in the hypothalamus, these animals are more dependent on the palatibility of the given food (KENNEDY 1953).

The activity studies showed that the fat mice were incapable of keeping their weights up when allowed to exercise. This is in accordance with the studies by MAYER (1955). He reported unchanged "spontaneous" activity in mice with goldthioglucose

obesity. In our work, however, on fully developed obesity the mice seemed to have a somewhat reduced "spontaneous" activity even when comparison with body weight times distance run was calculated. As soon as the fat mice by exercise or by their "spontaneous" activity in the rotating drums lost some weight, then a level of increased "spontaneous" activity supervened, which in turn led to a further weight reduction. This process went on until a limit was reached, where food intake and energy expenditure became equal. Some control mice showed no weight changes during the first days of running. Others did loose some weight, thus showing that animals of normal weight within the sedentary range are not always able to compensate for increased energy expenditures with increased food intake.

The recordings of water intake in the obese and normal animals showed that both groups of mice drank during feeding or after meals. One might therefore in this type of obesity exclude any fundamental damage to the mechanism regulating water intake.

Summary.

1. Eating and drinking behaviour have been studied in mice made obese by injection of goldthioglucose.

2. Continuous recordings of food and water intake showed that the obese mice ate less frequently but much larger average amounts of food compared to the control animals.

3. It is suggested that because of damage to structures in the ventromedial parts of the hypothalamus in the obese mice, these animals are more dependent on the peripheral mechanisms regulating food intake, such as that referable to the gastro-intestinal tract.

4. The obese mice were not able to compensate with increased food intake when kaolin as caloric inert material was added to the diet.

5. The control animals compensated for kaolin added to the diet by increasing the meal frequency or the amount of food eaten each time or by a combination of both.

6. The obese mice showed little ability to increase their food intake when forced to exercise or when exhibiting a progressively increasing "spontaneous" activity.

7. Continuous recordings of water consumption showed that no damage to the mechanisms regulating water intake was incurred in this type of obesity.

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Influence of Pyramidal Stimulation upon the Relay of Coarse Cutaneous Afferents in the Dorsal Horn.

By

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Recent results according to which spinal afferent transmission is inhibited via pathways descending from supraspinal levels (LINDBLOM and OTTOSSON 1953 a, 1954, 1956, HAGBARTH and KERR 1954) raise the question whether the pyramidal tract may transmit impulses influencing spinal sensory activity. Such a function may be suggested on the basis of histological evidence (SCHÄFER 1899, HOFF 1932, SZENTÁGOTHAI-SCHIMERT 1941) that pyramidal fibres end in the dorsal grey matter, where part of the afferent inflow is relayed. Electrophysiologically, LLOYD (1941 a) showed that appropriate stimulation at the pyramids activates the dorsal horn neurons not only in the external basilar region, where the descending impulses enter the grey matter, but even in the centre of the dorsal horn. HAGBARTH and KERR (1954) suggested from their experiments with bulbar stimulation that the pyramidal tract might participate in the descending influence on spinal afferent relays. As yet, however, conclusive evidence seems to be lacking.

The experiments to be reported were made during an investigation into the bulbar influence on the spinal cord dorsum potentials (LINDBLOM and OTTOSSON 1954, 1956). The N_1 deflection (BERNHARD 1953) was used as an index of transmission in the first synapse in the dorsal horn of impulses from coarse cutaneous afferents. It is shown that following stimulation restricted to the pyramids the N_1 deflection elicited from a cutaneous nerve

in the contralateral hindleg decreased in amplitude. An analogous effect was obtained by stimulation of the pericruciate area. Further, the results from cortical ablation experiments indicate that the effect of pyramidal stimulation is due to activation of descending fibres and not to antidromic activation of ascending fibre systems.

Methods.

The experiments were performed on decerebrate or nembutalized cats (20—40 mg/kg intravenously) which were given repeated small doses of D-tubocurarine and kept on artificial respiration. The pyramidal tract was stimulated by needle electrodes inserted into the pyramids from the floor of the fourth ventricle (positions controlled microscopically in frozen sections). The needles were lacquered except for the tip (0.1—0.2 mm), the insulation being checked microscopically and by resistance measurements. A second electrode was inserted in the neck muscles. The pad nerves or the sural nerves were stimulated bipolarly with single pulses of 0.1 msec. duration. The responses obtained from the lumbar cord dorsum were fed through a condensor-coupled differential amplifier with a time constant of one sec.

The results are based upon the observations from 18 needle tracks, distributed over the pyramidal tract crossection.

Results.

Effect of Pyramidal Stimulation.

Lumbar cord response. Fig. 1 A shows the lumbar cord dorsum response following stimulation of the bulbar pyramid with a train of 6 shocks (0.2 msec.) at 500 per sec. It consists of a slow negative wave with a duration of 20—30 msec. followed by a faint positivity. The response could never be elicited by less than three impulses with the parameters used and showed recruitment with increasing number of shocks (*cf.* LLOYD 1941 a). With six to seven impulses of low voltage a significant negativity was always obtained. As the beginning of the slow negative wave is not easily ascertainable, its latency, measured from the first stimulus artefact, can be calculated only approximately. 7 estimations in decerebrate cars have a range of 5—15 msec., mean 11 msec., while the "latency" to the top of the negative wave in the corresponding experiments has varied between 12—20 msec., mean 18 msec.

The response in Fig. 1 A has not been elicited from any other site in the bulb. When, *e. g.*, the stimulating electrode is in the

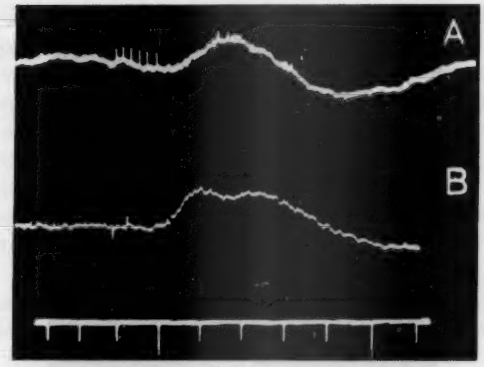

Fig. 1. Lumbar cord dorsum response to stimulation of, A, the pyramid with a train of six shocks of 0.2 msec. duration at 500 per sec. and 2 V; B, the pericruciate area with one shock of 4 msec. duration and 13 V. Time in 10 msec.

reticular formation outside the pyramidal tract the lumbar cord dorsum response is entirely different, consisting of a slow positive deflection with short latency preceded by two spike potentials (see LINDBLOM and OTTOSSON 1956). This fast response may also precede the slow negative pyramidal wave (Fig. 2 A) when the tip of the stimulating electrode is near the dorsal margin of the pyramid, or when the intensity of the pyramidal stimulus is so increased that it can be assumed that current spread is considerable.

Further evidence that the response in Fig. 1 A is not a response to activation of the reticular formation, collaterally or by stimulus spread, has been obtained from control experiments with medullary transection (*cf.* LLOYD 1941 a). Stimulation of the pyramid in a decerebrate cat gave the lumbar cord dorsum negativity at low voltages, and, at high voltages, a preceding positivity. The bulb was then transected at the level of the obex, caudally to the inserted needle, so that only the pyramidal tracts were left intact. Postsection pyramidal stimulation evoked the typical negative wave at the same low voltages whereas the positivity could not be elicited even at a very high stimulus strength. These experiments show that the slow negative wave in leads from the lumbar cord dorsum is a response to activation of the pyramidal tract.

Conditioning experiments. In a series of experiments the conditioning effect of the activity evoked from the pyramidal tract

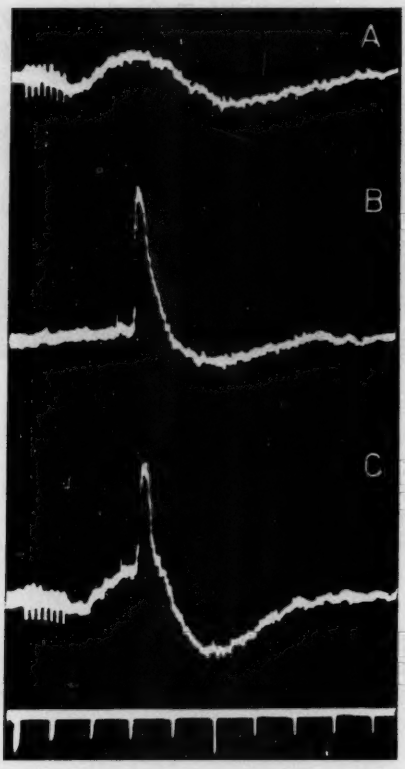

Fig. 2. Lumbar cord dorsum response to stimulation of, A, the pyramid; B, the contralateral pad nerve. Record C shows occlusion of the afferent response when the two responses are superimposed. Time in 10 msec.

on the lumbar cord dorsum response to stimulation of a cutaneous nerve in the hindleg was examined. Stimulation of one pyramidal tract regularly influenced the cord dorsum response evoked from a *contralateral* cutaneous nerve, which is illustrated by the conditioning experiment in Fig. 2. Record A shows the cord dorsum response evoked from the left pyramid, and record B the cord dorsum response following stimulation of low threshold fibres in the right pad nerve. In record C the latter response is superimposed on the negative wave of the pyramidal response. It is seen that the negative deflection (the N_1 deflection) elicited from the pad nerve is occluded by the negative wave evoked from the pyra-

midal tract. The reduction in amplitude of the N_1 deflection, superimposed on the top of the negative pyramidal wave, has varied between 15—30 per cent in different experiments. Maximal reduction was obtained on the top of the pyramidal negativity.

As a contrast the cord dorsum response evoked from an *ipsilateral* cutaneous nerve was not influenced following pyramidal stimulation, the N_1 deflection not being reduced in amplitude even when superimposed on the top of the slow negative wave.

Effect of Cortical Stimulation.

To localize the origin of the pyramidal fibres involved in the production of the slow negative wave, the cord dorsum responses elicited from different cortical areas were recorded and their influence was studied on the N_1 deflection in three experiments. Cortical stimulation was performed with bipolar surface electrodes with an interelectrodal distance of 2 mm and with single shocks of 1—4 msec. duration.

Stimulation of the pericruciate area evoked a complex cord dorsum response consisting of both positive and negative components. Following administration of small doses of nembutal the positive components are reduced and the remaining response consists of a slow negative wave with a latency of 12—15 msec. (Fig. 1 B). In conditioning experiments the contralateral N_1 deflection was reduced in amplitude by 20—30 per cent when it was elicited on the top of the slow negative wave, whereas the ipsilateral N_1 deflection only showed an insignificant decrease. Stimulation of other cortical areas gave responses with a dominating positive component and with short latencies and they had no significant conditioning effect on the N_1 deflection.

The results of the cortical stimulation indicate that at least part of the pyramidal tract fibres, the stimulation of which evokes the slow negative deflection on the cord dorsum, can be traced to the pericruciate area.

Effect of Pyramidal Stimulation after Unilateral Cortical Ablation.

The effects from pyramidal and cortical stimulation indicate that descending fibres influence neurons generating the N_1 deflection. However, as a response was recorded from the pyramid following peripheral stimulation and as evidence has been presented that the N_1 deflection signals activity in a spinobulbar transmission

system (BERNHARD and WIDÉN 1953) the possibility of antidromic activation of ascending fibres must also be considered. The pyramidal response to peripheral stimulation has been ascribed to activity in ascending, spinocortical, fibres, running in the pyramids (BRODAL and KAADA 1953, *cf.* BRODAL and WALBERG 1952). This interpretation has later been questioned by PATTON and AMASSIAN (1955) and LANDAU (1956) who assume that the pyramidal response represents volume spread of activity in overlying ascending tracts.

To exclude the relevance of the putative ascending pyramidal fibres for the effects described in this paper three experiments were performed in which a fairly large part of the left frontal area, including the motor cortex, was sucked out. After 2—4 weeks to allow the cortico-spinal fibres to degenerate, pyramidal stimulation was performed. It was found (i) that ascending responses could be recorded from both pyramidal tracts following stimulation of both pad nerves; (ii) stimulation in the right pyramid evoked a typical slow negative wave in leads from the cord dorsum which occluded the N_1 deflection elicited from the left sural nerve; (iii) stimulation in the left pyramid did not evoke negative activity at low voltages; on increasing the stimulus strength there appeared either positive activity, presumably due to stimulus spread to the reticular formation, or a negative wave occluding the N_1 deflection from the left nerve and consequently signalling stimulus spread to the right, intact, pyramid. The results of the degeneration experiments indicate that the slow negative wave following stimulation in the pyramids and the depressing effect on the N_1 deflection are due to activation of descending corticospinal fibres.

Discussion.

In the present investigation it was found that stimulation of the pyramidal tract at the medullary level evoked a slow negative response in leads from the lumbar cord dorsum. Section of the medulla caudal to the point stimulated, leaving only the pyramids intact, did not change the response, demonstrating that it was not due to activation of extrapyramidal structures. Spread of excitation to the reticular formation outside the pyramids was, in fact, indicated in the cord dorsum response by a preceding positive component (cf. LINDBLOM and OTTOSSON 1956). On

cortical stimulation a similar negative wave was obtained on the cord dorsum when the pericruciate area was stimulated indicating that at least part of the pyramidal fibres concerned originate in this area. In chronic experiments in which the frontal cortex had been excised, and the corticospinal neurons allowed to degenerate, pyramidal stimulation failed to evoke the typical negative wave on the cord dorsum. The inference from these observations is that the cord dorsum response to pyramidal stimulation is due to activation of descending cortico-spinal fibres, and not to backfiring in putative ascending spinocortical fibres in the bulbar pyramids.

In conditioning experiments a depression of the N_1 deflection was obtained which was maximal on the top of the slow cord dorsum negativity following pyramidal stimulation. The fact that the N_1 deflection was influenced only when elicited from a contralateral nerve is in agreement with the concept of a complete crossing of the pyramidal tract in the cat. The observation of a depression of the N_1 deflection following pyramidal stimulation leads to the conclusion that pyramidal fibres mediate a cortico-spinal inhibitory influence on the first synapse in the dorsal horn (cf. LINDBLOM and OTTOSSON 1953 b). The depression of the N_1 deflection is probably concerned with its post-synaptic part (see AUSTIN and MCCOUCH 1955, LINDBLOM and OTTOSSON 1956). Any further conclusions to be drawn from the present results are dependent upon what is known about the functional significance of the N_1 deflection and in regard to this the results obtained by BERNHARD and WIDÉN (1953) are of importance. These authors compared the N_1 deflection and the ventral root reflex response to stimulation of the same cutaneous nerve. When the stimulus strength was successively increased the reflex discharge continued to grow even when the N_1 deflection had reached its maximum. Further, in conditioning experiments the peripheral volley was followed by a reflex discharge though it was prevented from eliciting an N_1 deflection by an antidromic dorsal column volley. The lack of correlation between the polysynaptic reflex and the N_1 deflection was taken as indirect evidence that the N_1 deflection signals activity in a spino-bulbar transmission system. Thus the descending pyramidal activity described above would be concerned with the sensory inflow to higher levels. This interpretation is, however, not unequivocal, as long as the destination of the activity signalled by the N_1 deflection has not been directly es-

tablished, and it may also be too restricted. It may be difficult to dissociate between interneurons giving origin to ascending pathways from those mediating reflex activity (McCouch and Austin 1956).

The recruiting character and the slow time relations of the lumbar cord dorsum response to pyramidal stimulation indicates that it reflects internuncial activity. In fact, Lloyd (1941 a) has recorded a slow recruiting response directly from the spinal interneuron pools following similar stimulation. It does not seem possible from the present experiments to determine the nature of the pyramidal inhibitory influence on the N_1 deflection. It may be tempting to assume that the cord dorsum negativity elicited from the pyramids signals activity in the same neurons as those giving rise to the N_1 deflection, and consequently to interpret the conditioning effect as due to convergence of pyramidal fibres and coarse cutaneous afferents on the same neurons in the dorsal grey matter. The depression of the N_1 deflection may, however, also be due to specific inhibitory impulses in the same way as segmental interneurons in the intermediate horn may be inhibited from the pyramids (Lloyd 1941 a).

In a previous paper (Lindblom and Ottosson 1956) it was reported that monopolar stimulation of the bulbar reticular formation evokes a slow *positive* potential in leads from the lumbar cord dorsum and that activity of this kind, when evoked from certain reticular sites, has a depressing effect on the N_1 deflection. It can be questioned whether the two types of cord dorsum response — the positive reticular response and the negative pyramidal response — not only represent activity in two different systems but also reflect a different mechanism of influence upon the internuncial activity signalled by the N_1 deflection. However, the opposite polarity of the cord dorsum responses may be interpreted in other ways, *e. g.* on the basis of different orientation of the active neurons in relation to the recording electrode. In fact, the neurons connected to the pyramidal tracts seem to have a more dorsal localization than the neurons activated by reticulospinal fibres (cf. Lloyd 1941 a and b, 1944).

Summary.

1. In decerebrate or nembutalized cats whose pyramidal tracts were stimulated at the medullary level the typical response in leads from the dorsal surface of the

lumbar part of the spinal cord was a recruiting slow negative deflection and a subsequent positivity. The response was not influenced by a transverse section of the medulla caudal to the point stimulated, sparing only the pyramids.

2. A similar response could be elicited by stimulation of the pericruciate area in the cerebral cortex. When this area was sucked out and the pyramids were stimulated after an interval allowing the cortico-spinal fibres to degenerate, no response was obtained in leads from the lumbar cord dorsum.

3. In conditioning experiments the afferent cord dorsum response to low threshold stimulation of a cutaneous nerve in the hind leg (the N_1 deflection) was used as the test response. The N_1 deflection decreased in amplitude when superimposed on the slow negative wave elicited from the contralateral pyramid while it was not influenced following ipsilateral pyramidal stimulation. In an analogous way a depression of the N_1 deflection was obtained when the pericruciate area was stimulated. When the slow negative wave was not elicitable due to degeneration of the cortico-spinal fibres stimulation of the pyramid did not influence the N_1 deflection.

4. The results indicate that the pyramidal tract mediates a cortico-spinal inhibitory influence on the primary afferent relay in the spinal cord.

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Sensitization of the Submaxillary Gland above the Level Reached after Section of the Chorda Tympani.

By

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By injecting atropine repeatedly it is possible to create a supersensitivity to chemical stimuli in the submaxillary glands which resembles that caused by parasympathetic denervation (EMMELIN and MUREN 1950 a). The denervation is by necessity a preganglionic one whereas the atropine can be assumed to act by antagonizing the effect of the transmitter of the postganglionic neurone; from the "law of denervation" the latter type of sensitization could perhaps be expected to be more pronounced than the former one. It has not been possible to demonstrate such a difference with certainty (EMMELIN and MUREN 1951, EMMELIN 1952). It is, however, not easy to ascertain that a maximal degree of sensitization has been reached during atropine treatment. This is due to the wellknown tolerance to atropine which develops during continued administration of this drug. In an attempt to counteract this effect the doses of atropine were successively increased; but this possibility is limited since eventually lethal doses are approached. The high dosage of atropine creates a further source of error. The drug mostly used to test the level of sensitivity is adrenaline; in the course of the treatment a dose of atropine is soon reached which in the acute experiment renders the gland cells less sensitive to adrenaline (EMMELIN and MACINTOSH 1955).

It seemed desirable to use a parasympathicolytic agent which does not have the disadvantages of atropine. A more suitable drug was found in piperidino-aethyl-diphenyl-azetamid (Hoechst). According to SCHAUMANN and LINDNER (1951) this drug effectively inhibits salivary secretion induced by pilocarpine in rabbits. Some experiments on sensitization of salivary glands by this drug, for brevity called Hö 9980, have already been published (EMMELIN and HENRIKSSON 1953, STRÖMBLAD 1956 a and b). These experiments suggested that it is possible to increase the level of sensitization above that reached after section of the chorda tympani. In the present investigation the existence of such an additional sensitization has been confirmed, and the mechanism by which it is brought about has been analysed (section 4 of this paper). In order to be able to carry out such an analysis, however, it was necessary to study the pharmacological properties of the new drug in some detail. For this purpose acute experiments were carried out in which the parasympathicolytic action of Hö 9980 and its specificity was studied (section 1). Experiments are further described demonstrating that Hö 9980 is able to cause a supersensitivity by acting locally in the gland (section 2), and that it does not interfere with the release of acetylcholine from the parasympathetic nerve endings (section 3).

Methods.

Cats were used as experimental animals. In acute experiments chloralose was given as an anaesthetic. In most cases, however, repeated observations were made on the same animal in evipan anaesthesia using the method of EMMELIN and MUREN (1952). Adrenaline in different doses was given intracardially to induce secretion. The number of drops falling from the cannulae inserted into the submaxillary ducts were usually counted only; in some experiments the intervals between the drops were recorded with the ordinate recorder of CLEMENTZ and RYBERG (1949).

Further technical details will be given in the text below.

Results.

1. Acute Experiments.

In cats under chloralose the effects of increasing intravenous doses of Hö 9980[1] on the salivary secretion elicited by stimulation of the chorda tympani or the sympathetic nerve or by intravenous

[1] The drug was kindly supplied by A. B. WEBASS, Gothenburg.

injection of acetylcholine or adrenaline were investigated. A dose of 25 μg/kg Hö 9980, which did not dilate the pupil, was found to reduce the secretory effects of acetylcholine and chorda stimulation, and with 0.1 mg/kg the effects were completely abolished. These doses did not affect the secretory responses to adrenaline or sympathetic stimulation. Even the highest doses tested, about 12 mg/kg Hö 9980, did not change the secretory effects of adrenaline or sympathetic stimulation.

2. Sensitization by Injection of Hö 9980 into the Submaxillary Duct.

Previous experiments have shown that it is possible to affect the gland cells by drugs injected into the secretory duct, and that the effect within certain limits will remain local (EMMELIN, MUREN and STRÖMBLAD 1954). An attempt was made to sensitize the submaxillary gland by repeated injections of Hö. 9980 into the duct. The injections were made once a day in a brief ethyl chloride anaesthesia. Both submaxillary ducts were cannulated from the mouth using polyethylene tubes. In the right duct 1 mg of Hö 9980, dissolved in 0.1 ml of saline solution, was given,in the left gland 0.1 ml saline. The sensitivity of the glands was tested in evipan anaesthesia before the injections and after six injections. After this treatment the right but not the left gland was found to be supersensitive. Thus 5 μg/kg adrenaline injected intracardially produced before the treatment 4 drops of saliva on the right and 5 on the left side. After the treatment the right side gave 8 $^1/_4$ and the left 4 $^1/_4$ drops in response to 5 μg/kg adrenaline. On continued treatment the sensitivity rose further on the right side, but it started rising on the left side also, indicating that there was some absorption of the drug from the gland.

3. Release of Acetylcholine on Chorda Stimulation.

It seemed necessary to ascertain that Hö 9980 when given repeatedly to sensitize the gland cells does not interfere with the release of acetylcholine from the chorda terminals. A cat was treated with daily subcutaneous injections of Hö 9980, 1 mg/kg. After two weeks the cat was anaesthetized with chloralose. The glands were found to be highly sensitive towards adrenaline. One of the glands was then perfused with heparinized plasma containing eserine sulphate 10^{-5}. The acetylcholine content of the

venous effluent, during periods of rest and of stimulation of the chorda tympani, was estimated on the eviscerated, eserinized cat's blood pressure. The method corresponded in all technical details to that used by EMMELIN and MUREN (1950 b). Stimulation of the chorda was found to cause a release of acetylcholine. In one stimulation period the amount of acetylcholine was found to be 0.186 μg/kg (2 min. stimulation, 20 supramaximal stimuli/sec.). In a later period the amount was 0.256 μg/kg acetylcholine. Corresponding figures in animals which had not been treated with Hö 9980 were on the average 0.144 μg and 0.201 μg, respectively, according to the earlier investigation.

4. Level of Supersensitivity Reached.

When a dose of 1 mg/kg Hö 9980 was injected subcutaneously once a day a sensitization of the submaxillary gland towards adrenaline was regularly found to ensue. The sensitivity increased rapidly, following about the time course of the sensitization due to section of the chorda.

In the experiment of fig. 1 the sensitivity is expressed as secretory response to the standard dose of 5 μg/kg and in threshold dose of adrenaline. The right chorda was cut at the beginning of the experiment, and a supersensitivity was found to develop. Three weeks afterwards treatment with Hö 9980 was instituted and continued for eleven days. The sensitivity of the normally innervated gland started to rise and reached the level of the denervated gland. When the treatment was discontinued the sensitivity of the left gland decreased rapidly.

The supersensitivity produced by treatment with Hö 9980 was found to have the characteristics of that following section of the chorda:

a. The *threshold* was found to be lowered (fig. 1).

b. When doses of adrenaline causing submaximal responses were chosen the *rate of secretion* was found to be bigger than before the treatment.

c. Under these conditions the *duration of secretion* was found to be prolonged by the treatment.

Section of the chorda is known not to increase the *maximal rate of secretion*. Treatment with Hö 9980 was found not to affect the maximal secretory rate of the gland the chorda of which had been cut in advance. No comparisons could be made, in this

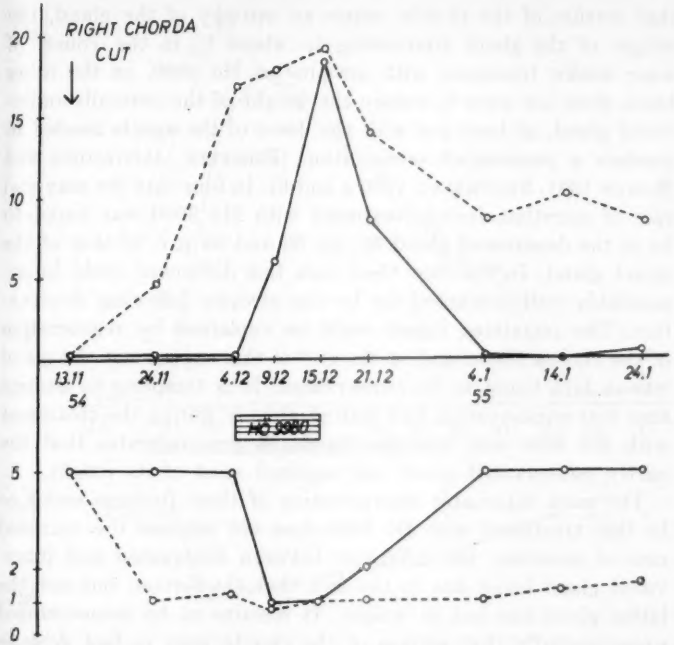

Fig. 1. Supersensitivity after section of right chorda-lingual nerve and treatment with Hö 9980. Broken line: right gland, solid line: left gland. Abscissa: time; ordinates: upwards the response in number of drops secreted on injection of 5 μg/kg adrenaline; downwards: threshold dose of adrenaline, μg/kg.

respect, between untreated and treated, normally innervated glands. This was due to the fact that the sensitivity of none of the glands of this series was high enough to allow an estimation of the maximal rate before treatment with Hö 9980; doses of adrenaline producing submaximal responses were found already to cause profound circulatory disturbances, which interfered with the estimation. Comparisons could, however, be made between the denervated and the innervated gland after treatment with Hö 9980. This was done in experiments in which the rate of flow was measured using a drop recorder.

These experiments showed that the maximal rate of secretion was higher in the innervated than in the denervated gland during treatment with Hö 9980. When looking for an explanation of this difference between the two glands it should be kept in mind

that section of the chorda causes an atrophy of the gland, the weight of the gland diminishing to about $^2/_3$ in the course of some weeks; treatment with atropine or Hö 9980, on the other hand, does not seem to reduce the weight of the normally innervated gland, at least not with the doses of the agents needed to produce a pronounced sensitization (EMMELIN, JACOBSOHN and MUREN 1951, STRÖMBLAD 1956 a and b). In four cats the maximal rate of secretion during treatment with Hö 9980 was found to be in the denervated gland 62, 65, 66 and 93 p. c. of that of the intact gland. In the first three cats this difference could be remarkably well accounted for by the atrophy following denervation. The remaining figure could be explained by regeneration of the chorda fibres; and at the end of this experiment the gland was in fact found to be reinnervated. It is tempting to assume that this reinnervation had started already during the treatment with Hö 9980 and that the figure 93 p. c. indicates that the partly reinnervated gland had regained most of its weight.

The most reasonable interpretation of these findings seems to be that treatment with Hö 9980 does not increase the maximal rate of secretion, the difference between denervated and innervated gland being due to the fact that the former, but not the latter gland has lost in weight. It remains to be demonstrated experimentally that section of the chorda does in fact *decrease* the maximal rate of secretion.

So far the similarities between the sensitization caused by treatment with Hö 9980 and those produced by section of the chorda have been emphasized. In fig. 1 it can be seen, however, that there was a slight tendency to increase in sensitivity, during treatment with Hö 9980, above the level reached after section of the chorda. It is possible that treatment for more than eleven days should have caused a further increase in sensitivity in this experiment. Treatment with Hö 9980 was given to 16 cats in this investigation; the period of treatment was usually longer than in the experiment of fig. 1. In all these cases there was an increase in sensitivity above the level produced by section of the chorda.

Fig. 2 illustrates one of these experiments. The right chorda-lingual nerve had been cut before the start of the experiment and the supersensitivity had reached a plateau. As can be seen, treatment with Hö 9980 caused a rise in sensitivity far above the level attained after denervation. This effect was obtained

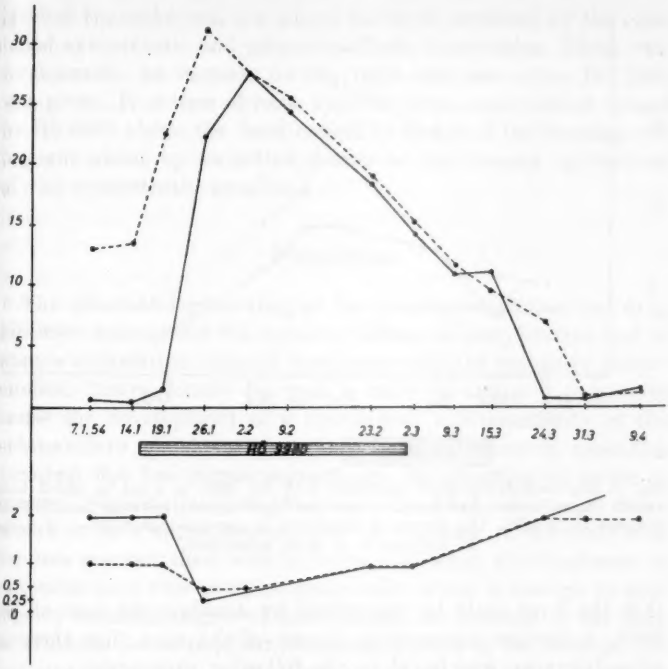

Fig. 2. For explanation see fig. 1. The right chorda-lingual nerve had been cut 8. 12. 1953.

in both glands, the rise of the denervated gland starting earlier than that of the contralateral one.

When the injection treatment was discontinued the sensitivity fell; after one week there was still some supersensitivity, but after two—three weeks the original level had been reached. In the experiment of fig. 2 the severed nerve had regenerated in the course of the treatment, and the sensitivity decreased to the level of an innervated gland.

In the experiment of fig. 2 the treatment with Hö 9980 was carried out for about one month and a half. During this long period there was a decrease in sensitivity on both sides. It was regularly found in these experiments that the sensitivity did not remain for several weeks at its high level but declined more or less rapidly. There was apparently some development of tolerance to Hö 9980 in the course of the treatment, because it was found

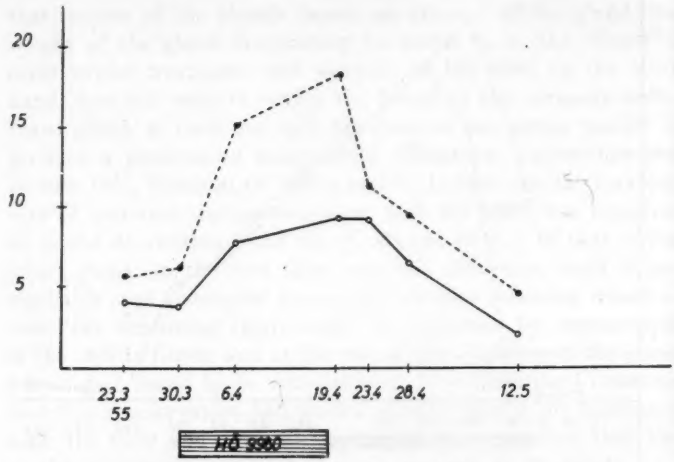

Fig. 3. Supersensitivity after treatment with Hö 9980 in a cat in which both chorda-lingual nerves had been cut and the right superior cervical ganglion extirpated 10. 3. 1955. The degree of sensitivity is expressed as number of drops in response to 2 μg/kg adrenaline.

that the level could be maintained by doubling the dose of Hö 9980. A further argument in favour of the view that there is some tolerance was found in the following experience.

In cats treated with Hö 9980 for some weeks acute experiments were made in chloralose anaesthesia. Stimulation of the chorda was found to cause a scanty flow of saliva. To suppress this response it was found to be necessary to use doses of Hö 9980 which were much bigger than those required in untreated animals.

Removal of the superior cervical ganglion is known to cause a supersensitivity, and this can be superimposed upon that caused by section of the chorda (EMMELIN and MUREN 1951). It could be hypothesized that treatment with Hö 9980 causes a combination of both these types of supersensitivity. In a series of experiments the chorda-lingual nerve was cut on both sides and the right superior cervical ganglion extirpated. Fig. 3 shows the outcome of one of these experiments. The operation had been carried out two weeks before the tracings of fig. 3 start. A constant level of supersensitivity had been reached, the level of the right gland being the higher one; treatment with Hö 9980 increased the sensitivity of the left gland above the pretreatment

level of the right one, *i. e.* above the level produced by the combined sympathetic and parasympathetic denervation. There was, furthermore, an increase on the right side also when Hö 9980 was given. It is thus obvious that the extra sensitization caused by Hö 9980 above the level caused by section of the chorda is not brought about by an action similar to that caused by removal of the sympathetic ganglion.

Discussion.

The pharmacological analysis has demonstrated that the drug Hö 9980 antagonizes the secretory effects of acetylcholine and of chorda stimulation without interfering with the release of acetylcholine. Acting locally by such a mode of action it is able to cause the development of a pronounced supersensitivity of the submaxillary gland to adrenaline. In this respect it resembles atropine but has certain advantages. Its duration of action is longer, necessitating less frequent injections. Although there seems to be a slight development of tolerance to Hö 9980 it is far less marked than with atropine and when the treatment is extended over two or three weeks only, which is enough to produce a maximal degree of sensitization, no increase in the dose of Hö 9980 seems required. Reasonable doses of Hö 9980 do not antagonize the secretory effect of adrenaline whereas during treatment with atropine an antagonistic concentration is attained. This antagonistic effect of atropine, and the successively developing tolerance render it difficult to find out the highest possible level of sensitivity that can be produced by atropinization. In some experiments with atropine there has in fact been some indication that a level can be obtained which exceeds that produced by section of the chorda (Emmelin and MacIntosh 1955). Using the drug Hö 9980 we have regularly surpassed the chorda supersensitivity level.

The fact that "atropinization" can give a higher degree of sensitivity than section of the chorda could be explained in different ways:

1. It might be that the section of the nerve has been incomplete, and that the "atropinization" has annulled the action of some remaining secretory fibres. We have, however, been aware of this possible source of error and cut the chorda-lingual nerve far central to the region where the chorda fibres start to branch off.

2. Section of the chorda can be supposed to affect not only secretory but vasodilator fibres as well; atropine, on the other hand, does not annul the vasodilatation caused by stimulation of the chorda, and we have made sure that this is true for Hö 9980 also. This could possibly explain the difference in sensitivity level found after chorda section and "atropinization"; it should then be assumed that the secretory effect of adrenaline should be diminished because of vasoconstriction of supersensitive vessels in denervated, but not in "atropinized" glands. We have, however, been unable to demonstrate convincingly a pronounced sensitization of the vessels towards adrenaline after section of the chorda. It may further be recalled that the threshold value of adrenaline is found to be lower after "atropinization" than after denervation, and such an effect could surely be explained on a vascular basis only if the vascular sensitization were very pronounced.

3. The possibility that Hö 9980, apart from its atropine-like effect, possesses some ganglionic blocking or sympathicolytic action, through which an additional supersensitivity could be called forth by antagonising an activity in the sympathetic secretory fibres, was experimentally excluded. According to the acute experiments Hö 9980 is devoid of such actions even in big doses. Furthermore, it was found that after section of the chorda and removal of the sympathetic ganglion treatment with Hö 9980 is still followed by an increased sensitivity.

4. In our first experiments we had the impression that treatment with Hö 9980 increases the maximal rate of secretion, and were apt to attribute the additional supersensitivity to some unknown pharmacological action of the drug causing an increase in the "reactivity" of the gland. This is the reason why a special series of experiments was carried out using ordinate recorders to measure the maximal rate of secretion more accurately. The conclusion from these experiments was, however, that Hö 9980 does not increase the maximal rate of secretion, as has been discussed above.

5. The sensitization caused by Hö 9980 resembled in fact very closely that produced by section of the chorda, apart from the fact that it was more pronounced. It seems reasonable to assume that both these sensitizations have ultimately the same mechanism: in both cases the gland cells are deprived of the sialogogue action of acetylcholine. The quantitative difference between the

two sensitizations would then be due to the fact that section of the chorda leaves some cholinergic mechanism which is still able to act on the gland cells, to some degree counteracting the development of the supersensitivity. Such a mechanism could obviously be provided by the postganglionic parasympathetic neurone, which is left intact when the chorda is severed. Treatment with Hö 9980 or atropine should then mean a more complete "denervation" of the gland than preganglionic section of the chorda, antagonizing the effect of acetylcholine released in an active postganglionic neurone or leaking from its terminals. Generally, this would agree with Cannon's law of denervation, according to which the sensitization is most pronounced in the structure directly denervated.

According to this conception section of the chorda should cause a sensitization by depriving the gland of secretory impulses from the central nervous system, mediated by acetylcholine. "Atropinization" should have the same effect and in addition deprive the gland of the action of acetylcholine derived from the postganglionic parasympathetic neurone; this should cause an additional sensitization. Even "atropinization" should not create the highest degree of supersensitivity possible. The presence of the sympathetic innervation should still counteract a complete sensitization, which, however, should be attained after removal of the superior cervical ganglion in an "atropinized" cat.

Summary.

After repeated subcutaneous injections of Hö 9980, a drug with pronounced atropine-like properties, a supersensitivity towards adrenaline develops in the submaxillary glands of cats. The supersensitivity closely resembles that produced by section of the chorda tympani but is more marked. Evidence is given to show that the supersensitivity caused by the drug is entirely due to the parasympathicolytic activity, and it is concluded that the sensitization above the level produced by the preganglionic denervation is due to exclusion of some action of the parasympathetic postganglionic neurone on the gland cells.

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Volume Conduction of the Spike of the Motor Unit Potential Investigated with a New Type of Multielectrode.

By

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Motor unit potentials usually contain diphasic components of short duration and high amplitude (Fig. 1 a). In the present study the spread of these spikes, *i.e.* their volume conduction, was measured in the human brachial biceps in a plane at right angles to the fibre direction by recording the variation in spike amplitude and duration along a multielectrode inserted transversely to the muscle fibres. The results were treated in terms of volume conduction analysis and an estimate was made of the number of muscle fibres which contribute to the spike of the motor unit potential.

Methods.

The motor unit potentials were recorded during voluntary contraction at slight or moderate effort, the discharge frequency being 10 to 15 per sec. In a special series of experiments action potentials were initiated by local electrical stimulation through a bipolar electrode inserted in the distal part of the muscle (BUCHTHAL, GULD and ROSENFALCK 1955 a).

The measurements of the spike were essentially confined to its positive-negative deflection of short duration illustrated at high sweep speed in Fig. 2 a. The amplitude was defined as the difference between the highest positive and negative deflections. Only those spikes were included in the volume conduction study which at the lead of the multielectrode with maximum response had a linear slope such as to indicate that they were composed solely of one spike. When in other leads a sudden change in slope of the steep deflection indicated the superposition of a second spike (Fig. 2 b), the amplitude was measured

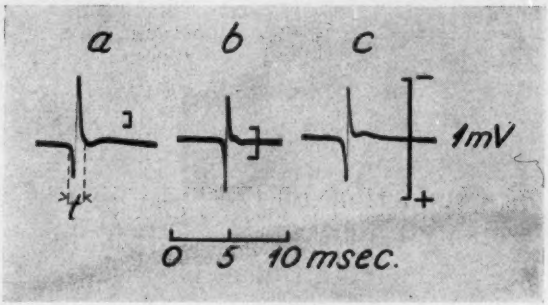

Fig. 1. (a) motor unit potential, (b) potential evoked by local electrical stimulation at threshold intensity, (c) fibrillation potential from denervated muscle (human brachial biceps, 36.5° C).

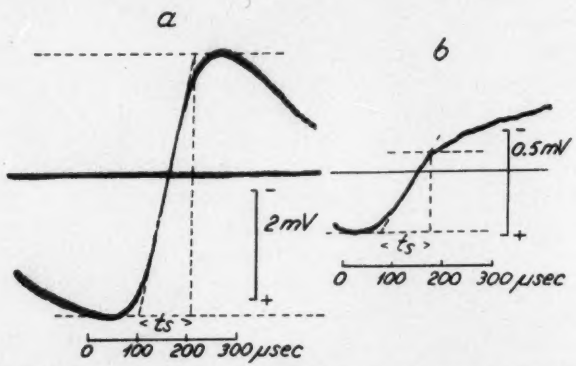

Fig. 2. To illustrate the measurements of the duration (t_s) and amplitude of the short positive-negative deflection of a spike potential. (a) without and (b) with change in slope.

of that portion of the steep deflection which was synchronous with the deflection recorded at a lead where there was no change in slope.

The *duration* (t_s, Fig. 2) of the positive-negative deflection was determined as the time interval between the points at which the extrapolated steep deflection intersected the two horizontal lines at the level of the positive and negative maxima of the potential.

The Multielectrode.

Analysis of the spread of the individual spike potential over the cross section of the muscle required leading off with a suitable number of electrodes placed at well-defined distances from each other. For this purpose a special multielectrode was constructed

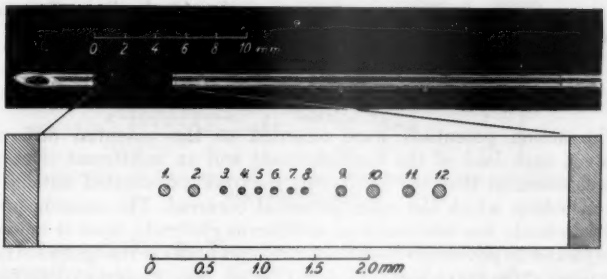

Fig. 3. Multielectrode containing twelve leads 50 or 100 μ in diameter; for lead 3 to lead 8, the centres of the leading-off areas were 0.15 mm apart, for lead 1 to lead 3 and lead 8 to lead 12, the centres were 0.30 mm apart.

which contained twelve separate leads placed along a stainless steel cannula one mm in diameter[1]. A section of the cannula metal was replaced by heat hardened Araldite in which the 12 platinum electrodes were embedded over a length of 2.5 mm (Fig. 3). Six of these electrodes had a circular leading-off area of 50 μ in diameter and a distance between centres of 0.15 mm. The six other electrodes had a leading-off area of 100 μ in diameter and a distance of 0.3 mm between centres.

Electrode Noise.

When placed within the muscle or in a 0.9 per cent sodium chloride solution, the flicker and the shot noise of the untreated leading-off surfaces of the multielectrode were very high, 3—15 μV r.m.s. In addition numerous artifact potentials with an amplitude of up to several millivolts appeared. They were negative, had a sawtooth shape and a duration of 1—10 msec and occurred with a frequency of 1—1,000 per sec.

The noise could be substantially reduced by placing the electrode in a 0.9 per cent sodium chloride solution and passing a 2 ma current through a large platinum electrode (anode) to each leading-off surface (cathode) for at least ten seconds, until small gas bubbles developed at the leading-off surface of the multielectrode. By this procedure the electrode noise was diminished to 2—3 μV r.m.s., so that it only slightly exceeded the noise of the amplifier (1.5 μV r.m.s.). Furthermore, the artifact potentials could be entirely abolished, provided that the currents which passed through the electrode from grid current and cable leaks were below 10^{-9} amp. The electrolytic treatment of the electrode had to be repeated about once or twice a month.

The multielectrode was sterilized for twenty minutes in water at 100°C. It was inserted into the muscle outside the innervation zone

[1] The multielectrodes are manufactured by DISA Elektronik, Copenhagen.

and turned around its axis in such a way that the leading-off surfaces were situated as close as possible to the active muscle fibres.

The Indifferent Electrode.

The action potentials were recorded as the potential difference between each lead of the multielectrode and an indifferent electrode. It was essential that the indifferent electrode be situated outside the region within which the spike potential occurred. The cannula of the multielectrode was unsuitable as indifferent electrode, since it unavoidably picked up potentials from the motor unit under investigation. Therefore, one of the outer leads, 1 or 12 (Fig. 3), was chosen as indifferent electrode. In experiments with various electrode distances it was ascertained that with the maximum spike amplitude recorded on lead 5 or 6 of the multielectrode, the distance to leads 1 and 12 was such that they were situated outside the region within which the spike was recorded. As indifferent electrode the one of these two leads (1 or 12) was chosen which showed the least interference from potentials of other fibres of the motor unit. Potentials sometimes appeared with approximately the same amplitude on all the leads of the multielectrode. They had a reversed sign, the steep deflection being first negative — then positive, while ordinarily it is first positive then negative. Such potentials were picked up by the indifferent electrode and disappeared from most of the electrodes when the site of the multielectrode was slightly changed.

Multielectrode Switch and Amplifiers.

A three channel DISA electromyograph was used and when high resolution in time was desired, its amplifiers were connected to a dual beam Dumont Oscilloscope (Type 322). The lead of the multielectrode which recorded the largest potential, usually lead 5 or 6, was kept in constant connection with one channel of the electromyograph. The potential picked up by this lead served to ascertain that recording occurred from the same motor unit throughout and made it possible to evaluate time relations between the potentials recorded on the different leads. The other leads of the electrode were successively switched to the two other channels. A 50 μsec square wave pulse was introduced as time reference on the three cathode ray tubes of the electromyograph.

Each lead of the multielectrode could be connected by a 40 cm cable via a switch to the input of the amplifiers. Since the cable connecting the multielectrode and the switch had to be both screened and thin and flexible, it had a relatively high capacity (500 $\mu\mu$F per m). The effect of cable capacity and of leakage between screen and core were reduced by connecting the screen of each cable to the cathode follower which was in parallel with the input of the amplifier.

When switching from one lead of the multielectrode to another, the screen connected to the cathode of the cathode follower was

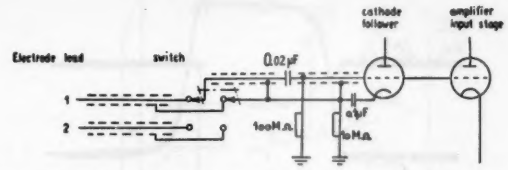

Fig. 4. Connection of the leads of the multielectrode to the amplifiers of the electromyograph.

switched as well (Fig. 4). The screen of the cable was capacity-coupled to the cathode to prevent direct current flow through the electrodes from the d.c. cathode potentials via a leakage between screen and core in the electrode cable. All twelve cables were surrounded by a common screen which was connected to earth.

The *frequency band of the recording device* was measured as previously described (BUCHTHAL, GULD and ROSENFALCK, 1954 b) with the electrode inserted into the muscle. Thereby consideration was taken of the voltage division between electrode impedance and amplifier input impedance.

The recording of duration and amplitude of the approximately 100 μsec positive-negative deflection of the action potentials required a sufficiently short *rise time* of the amplifier. With sinusoidal input the *upper limiting frequency* of amplifier plus electrodes defined by three db discrimination was 8,000 cps, *i.e.* slightly below that of the amplifier itself (12,000 cps). Determined by means of square wave pulses introduced into the amplifier via the multielectrode in saline the rise time was 16—20 μsec (Fig. 5). Calculations similar to those made by SOLMS, NASTUK and ALEXANDER (1953) gave with a rising time of 20 μsec an error of 6 per cent in the duration of the positive-negative deflection of the motor unit spike and an error of 1 per cent in its amplitude.

Recording.

The potentials were recorded on photographic paper or film. A time base for the single sweep of 30 μsec per mm was used for analysis of the positive-negative deflection. The film speed was 25 cm per sec. The beams of the oscilloscope were triggered by the initial positive deflection of the spike. In this way all discharges were recorded, thereby saving photographic film and reducing the time over which a constant effort had to be maintained by the subject. When recording the total action potential a time base of 0.5 or 1 msec per mm, a sweep frequency of five per sec, and a paper speed of five cm per sec were used.

Subjects.

The experiments were performed on the brachial biceps of five student volunteers, 20 to 25 years of age, without signs or symptoms

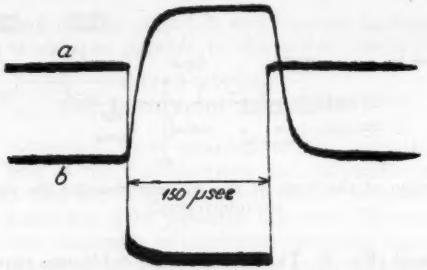

Fig. 5. Distortion of a 150 μsec square wave pulse (a) through a 50 μ electrode in saline connected to the input of the amplifier (b). Rising time = 16 μsec.

of neuromuscular disorders. The intramuscular temperature was 36—37° C.

Results.

Spike Amplitude and Duration as a Function of Distance

For a study of the spike amplitude as a function of the distance between the potential source and the recording electrode, a response was searched for on lead 5 or 6 of the multielectrode with high amplitude and short duration. Thereby the smallest possible distance from the active fibre or fibres was insured. By suitable electrode placement the action potential amplitude recorded with this type of electrode was up to 10 mV. This amplitude is considerably higher than the maximum amplitudes recorded with ordinary concentric electrodes (4.2 mV, leading-off surface 0.1 × 0.4 mm; BUCHTHAL, GULD and ROSENFALCK 1954 b). The difference is due to the smaller leading-off surface in the case of the multielectrode. The diameter of the 50 μ leading-off surfaces of the multielectrode is about the same as the diameter of the muscle fibres. Therefore, a further decrease in the leading-off area would give only a slight further increase in maximum amplitude (HÅKANSSON 1956). The 100 μ leading-off surfaces were the outer leads of the multielectrode and therefore at such a distance from the potential source that the amplitude recorded by them was not significantly distorted by their area.

With increasing distance along the multielectrode from the lead with maximum response the spike amplitude decreased steeply. In Fig. 6 an example is given of this decrease recorded with

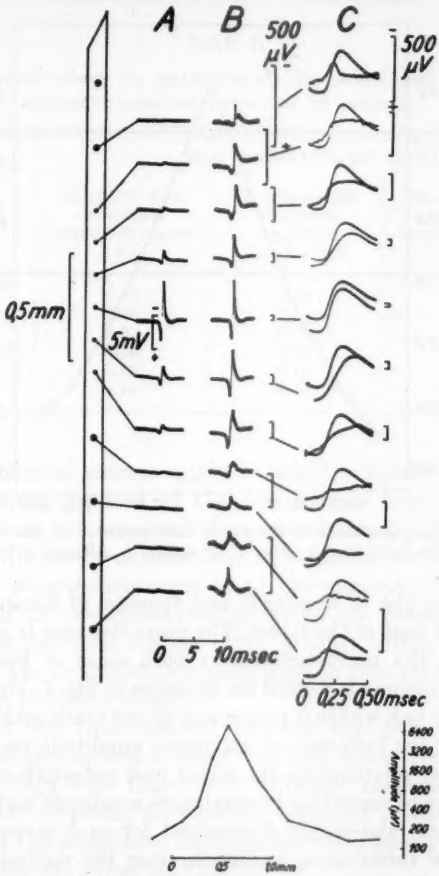

Fig. 6. The spike of a motor unit potential as recorded from different leads of the multielectrode.

A. The spike potential recorded with the same amplification on all leads.
B. The same potential as A as recorded with adjusted amplification.
C. The same potential as A and B as recorded at high sweep speed and with adjusted amplification for a detailed study of the positive negative deflection. For comparison the potential from lead 6 was recorded simultaneously on the other beam of the oscilloscope.

The curve below shows the relationship between spike amplitude and distance along the multielectrode. *Ordinate:* Spike amplitude in μV, logarithmic scale. *Abscissa:* Distance along the multielectrode in mm.

constant (A) and with adjusted amplification with a time base of 0.5 msec per mm (B); and with a time base of 30 μsec per

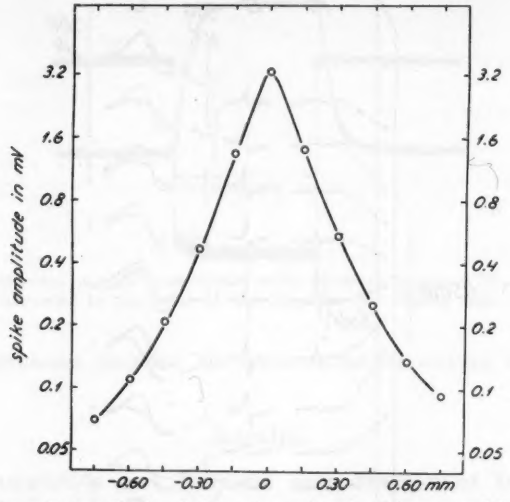

Fig. 7. The spike amplitude of the motor unit potential as recorded along the multielectrode (mean value of 58 spikes, 5 different subjects.)

mm (C). The plot of amplitude as a function of distance is shown in the lower part of the figure. The mean decrease in spike amplitude along the multielectrode to both sides of the lead with maximum response is plotted for 58 spikes in Fig. 7. The amplitude decreased to half within 0.12 mm and to one tenth within 0.38 mm.

To study the influence of maximum amplitude on this amplitude-distance relationship, the motor unit potentials were divided in three groups according to maximum amplitude and the amplitude-distance relationship determined for each group (Table 1). The relative variation in amplitude along the multielectrode was slightly smaller for potentials with low than for potentials with high maximum amplitude. The different amplitude-distance relationships were used to analyse the volume conduction of the spike and to estimate the number of muscle fibres contributing to the spike of the motor unit potential (cf. p. 346).

In many instances two spikes were recorded from the same motor unit, within the small region scanned by the multielectrode. In fifteen experiments the site of the maximum amplitude for the two spikes could be determined. The average distance between the leads with the maximum response for the respective spikes was 0.7 mm (0.3 to 1.05 mm). The distance between the corre-

Table 1.

Spike amplitude along the multielectrode for motor unit spikes with different maximum amplitude (5 subjects).

Distance from point of maximum amplitude mm	Mean amplitude in mV for		
	12 spikes with maximum amplitude above 5 mV	13 spikes with maximum amplitude 2.5—5.0 mV	33 spikes with maximum amplitude below 2.5 mV
0	8.40	3.40	1.35
0.15	2.99	1.63	0.69
0.30	0.87	0.65	0.31
0.45	0.36	0.30	0.16
0.60	0.19	0.15	0.09
0.75	0.14	0.10	0.06

sponding potential sources might be slightly higher on account of the different distance of the two sources from the multielectrode. From the amplitude-distance relationship (Fig. 7) this increase in distance was estimated to be no more than ten per cent of the distance measured along the electrode. The spatial separation of the two spikes corresponds to the distance between neighbouring potential sources (fibre groups) within the active motor unit. The two spikes were displaced in time on the average by 1.3 msec (0.5—2.3 msec).

Sometimes the time interval between the positive-negative deflections of two different spikes was only about 100 μsec, and unless recordings were taken with sufficient resolution in time the two spikes composing the potential could not be discriminated. Examples of the amplitude variation for two spikes slightly displaced in time are given in Fig. 8.

The *duration* (t_s) of the steep positive-negative deflection, as defined in Fig. 2 was determined for all those motor unit potentials which were recorded at high sweep speed. It averaged at maximum amplitude 136 μsec (s.d. 36 μsec, 32 potentials). With increasing distance along the multielectrode from the lead with maximum response the duration increased; at a distance of 0.38 mm where amplitude was reduced to one tenth of its maximum value, the duration was increased by 100 μsec. Potentials with different maximum amplitude had the same average enhancement in duration (For an estimation of the accuracy of the measurement of these short durations, see p. 335).

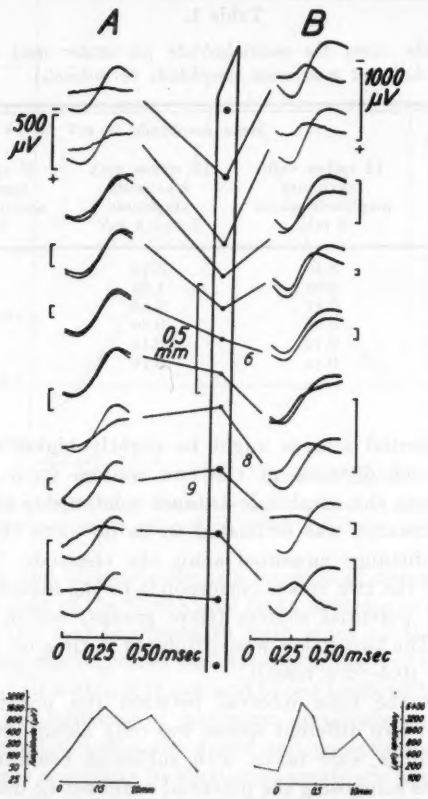

Fig. 8. Two examples (A and B) of motor unit potentials each containing two spikes slightly displaced in time.
In the lead marked 9 (A) and in the lead marked 8 (B), a spike occurred about 100 µsec earlier than the spike recorded as time reference on the lead marked 6. This potential was recorded simultaneously on the other beam of the oscilloscope. The curves below show the relationship between spike amplitude and distance along the multielectrode. The shape of the curves suggests the complexity of the spike as revealed by high speed recording only. *Ordinate:* Spike amplitude in µV, logarithmic scale. *Abscissa:* Distance in mm along the multielectrode.

Comparison with Spike Potentials Elicited by Electrical Stimulation and with Fibrillation Potentials.

The main component of the all-or-none action potentials elicited with local electrical stimulation at threshold intensity (BUCHTHAL, GULD and ROSENFALCK 1955 a) and that of fibrilla-

Table 2.

Volume conduction and duration of the spike of the motor unit potential and of electrically evoked potentials, total action potential duration and the duration of fibrillation potentials (36.5° C).

	Distance along multielectrode to $^1/_{10}$ of maximum amplitude mm	Per cent increase in duration of steep deflection at $^1/_{10}$ of maximum amplitude	Spike duration		Total action potential duration msec
			positive-negative deflection μsec	positive phase plus negative phase msec	
motor unit potentials	0.38	75	136 s.d. 36[1] (N = 32)	1.42 s.d. 0.40[1] (N = 31)	8.7 s.d. 1.0[2] (N = 275)
electrically evoked potentials	0.55	50—100	156 s.d. 72[1] (N = 13)	1.40 s.d. 0.53[2] (N = 11)	3.5 s.d. 0.8[2] (N = 11)
fibrillation potentials from denervated muscle	—	—	about 150[2]	1.66 s.d. 0.55[2] (N = 51)	2.45 s.d. 1.2[2] (N = 118)

N = number of potentials; s.d. = standard deviation.
[1] Measured at multielectrode lead with maximum response.
[2] Randomly recorded with concentric electrode.

tion potentials (DENNY-BROWN and PENNYBACKER 1938) is a diphasic spike (Fig. 1 b and c). In Table 2 data are given on the change in duration and amplitude of the positive-negative deflection as a function of distance for motor unit and for electrically evoked potentials. The table contains, furthermore, the duration of the positive-negative deflection, the spike duration and the total action potential duration of motor unit potentials, of electrically evoked potentials and of fibrillation potentials. The positive-negative deflection of the spike of electrically evoked potentials and of fibrillation potentials had a duration of the same order of magnitude as that of the spike of motor unit potentials. In *electrically evoked potentials* at threshold stimulation maximum amplitudes of up to 12 mV were recorded with suitable electrode placement, *i.e.* the same as the maximum values for motor unit spikes. Their duration increased and their amplitude decreased with increasing distance from the multielectrode lead with maximum response in the same way as for the steep deflection in the spike of motor unit potentials. *Fibrillation potentials*

were only studied by means of conventional concentric electrodes. Their maximum amplitude was 1.0 mV.

The *spike duration* was defined as the duration of the initial positive plus the subsequent negative deflection (t in Fig. 1 a). This duration was delineable in the motor unit potential recorded at the multielectrode lead with maximum response. It was of the same order of magnitude as that of the spike of electrically evoked and of fibrillation potentials (Table 2). The *total duration* of the motor unit potential as obtained by random sampling with concentric electrodes was about six times the spike duration. The total duration of electrically evoked and of fibrillation potentials was 2.5 and 1.5 times the spike duration. Hence, the long duration of the motor unit potential must be due to superposition of temporally dispersed activity from more distant parts of the motor unit which give rise to a protracted initial and terminal portion of the potential.

Volume Conduction of the Spike of the Motor Unit Potential.

The purpose of the treatment of the present data in terms of volume conduction analysis was to compare the measurements obtained on the spike of the motor unit potential with those from isolated muscle fibres (HÅKANSSON 1957) and with LORENTE DE NÓ's (1947) calculations of the spread of the nerve action potential. The logarithm of the amplitude of action potentials from isolated muscle fibres immersed in a volume conductor decreases approximately rectilinearly with the logarithm of the distance from the fibre axis. Furthermore, the relative reduction in amplitude with increasing distance is the same for fibres of different radius as it appears from the closely parallel course of curves 1, 2 and 3 in Fig. 9.

According to LORENTE DE NÓ (1947) the volume conducted action potential from a nerve can be calculated from the monophasic action potential recorded from the surface of the nerve when surrounded by an insulating medium. The potential φ in a point P (r, z_1) of an isotropic conductor is with good approximation determined by:

$$\varphi(P) = -\frac{A\beta}{4\pi}\int_{-\infty}^{\infty}\frac{\delta^2 V_e}{\delta z^2}\cdot\frac{dz}{\sqrt{r^2+(z-z_1)^2}} \quad \ldots\ldots\ldots (1)$$

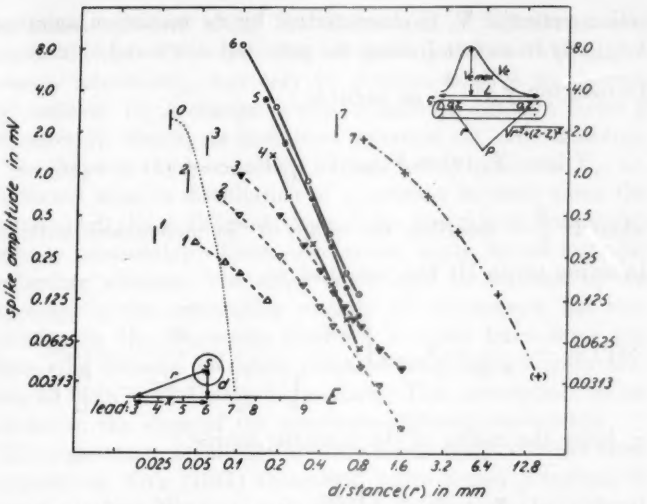

Fig. 9. Spike amplitude as a function of distance (r) from the axis of the potential source, both on a logarithmic scale.

1—3: Isolated frog muscle fibre in Ringer's solution (HÅKANSSON, 1957).
(1) fibre radius 23.5 μ
(2) fibre radius 41 μ
(3) fibre radius 56 μ
The vertical lines indicate the site of the fibre surface.
4—6: Motor unit spikes, human brachial biceps. (Table 1.)
(4) maximum spike amplitude below 2.5 mV
(5) maximum spike amplitude 2.5—5 mV
(6) maximum spike amplitude above 5 mV
(7) *Whole frog nerve*, radius 0.5 mm, constructed from LORENTE DE NÓ's (1947) theoretical action potentials, with the amplitude in arbitrary units. The vertical line indicates the site of the nerve surface.
(0) *Single spinal ganglion cell*, radius 30 μ (constructed from SVAETICHIN 1951).
Ordinate in mV for curves 1 to 6 and in arbitrary units for curves 7 and 0.
Upper inset: To illustrate the calculation of the potential in point P at distance r from the axis z of a cylindrical potential source with radius r_s. V_e is the monophasic surface potential as recorded in an insulating medium. L is the extent of the action currents along the source, and $V_{e\,max}$ the maximum amplitude of the monophasic action potential.
Lower inset: To show the transformation of distances (x) along the multielectrode to distances (r) from the axis of the potential source (S) with (d) as the shortest distance between multielectrode and source.

where V_e is the monophasic potential, z the distance along the axis of the nerve, r the distance of P from the axis, z_1 its position along the axis, and A the cross sectional area of the nerve (upper inset Fig. 9). The analysis of LORENTE DE NÓ can be applied to potential sources other than nerve. The monophasic surface

action potential V_e is characterized by its maximum amplitude $V_{e\,max}$, by its extent L along the potential source and by its shape. Introducing $y = \frac{z}{L}$ as variable

$$V_e(y) = V_{e\,max}\, f\left(\frac{z}{L}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where $f\left(\frac{z}{L}\right)$ describes the shape of the monophasic potential. Inserting (2) in (1) the potential is

$$\varphi(P) = -\frac{r_0^2}{4\,L^2} \cdot V_{e\,max} \int_{-\infty}^{\infty} \frac{\delta^2 f}{\delta y^2} \cdot \frac{dy}{\sqrt{\left(\frac{r}{L}\right)^2 + \left(y - \frac{z_1}{L}\right)^2}} \quad \ldots (3)$$

r_0 being the radius of the potential source.

$$\text{With } g\left(\frac{r}{L}, \frac{z_1}{L}\right) = \int_{-\infty}^{\infty} \frac{\delta^2 f}{\delta y^2} \cdot \frac{dy}{\sqrt{\left(\frac{r}{L}\right)^2 + \left(y - \frac{z_1}{L}\right)^2}} \quad \ldots (4)$$

the potential around the source is given by:

$$\varphi(P) = -\frac{r_0^2}{4\,L^2}\, V_{e\,max} \cdot g\left(\frac{r}{L}, \frac{z_1}{L}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

From (5) the following conclusions can be drawn as to the amplitude-distance relationship of different potential sources in an isotropic conductor: (1) An increase in the radius r_0 of the source causes a parallel displacement of the logarithmic amplitude-distance relationship to higher values of amplitude, the amplitude being proportional with r_0^2. (2) If the spread of the action currents (L) along the source is increased, the amplitude-distance relationship is displaced to proportionally higher values of the distance, the factor g in equation (5) being a function of $\frac{r}{L}$. In addition, an increase in L causes a parallel displacement of the amplitude-distance relationship to lower amplitudes, the amplitude being proportional to $\frac{1}{L^2}$. A further displacement in amplitude could arise from differences in the maximum amplitude of the monophasic potential. Thus, changes in radius, in spread of

the action currents along the source and in maximum amplitude do not affect the slope of the logarithmic amplitude-distance relationship, but only its position relative to abscissa and ordinate. (3) A change in slope requires a different factor g (equation 5). Hence, as seen from equation (4), only a change in the shape of the monophasic surface action potential V_o, *i.e.* a different relative distribution of membrane currents along the source, results in a different *slope* of the logarithmic amplitude-distance relationship. These conclusions apply to an isotropic conducting medium. The slope might also be affected by an anisotropy in the surrounding medium. If, for example, the conductivity in the transverse direction is lower than along the source, the decrease in action potential amplitude is more pronounced than in the isotropic medium. This corresponds to an increase in the slope of the amplitude-distance relationship.

The logarithmic amplitude-distance relationship obtained from LORENTE DE NÓ's (1947) theoretical nerve action potentials is approximately rectilinear and parallel to that of the isolated muscle fibres (Fig. 9, curve 7). It follows from equation (5) that the relationship for nerve is displaced to larger distances on account of the larger extent of action current spread and the larger diameter of the potential source in nerve as compared with isolated muscle fibres.

The application of a similar analysis to the volume conduction of the spike of the motor unit potential was complicated by the fact that the leads of the multielectrode were situated along a line (E) with an unknown distance (d) from the axis (S) of the potential source (Fig. 9, lower inset). A direct determination of the distance (d) by recording the spike potential along two multielectrodes inserted at right angles to each other turned out not to be possible. The mutual position of the two multielectrodes could not be determined with the degree of accuracy necessary in view of the marked change in spike amplitude with distance from the potential source. However, the distance (d) could be estimated indirectly by assuming that the amplitude-distance relationships of different motor unit spikes are parallel when amplitude and distance are plotted on logarithmic scales. This assumption is based on the experimental findings in isolated muscle fibres of different radius (Fig. 9 curves 1—3 constructed from HÅKANSSON 1957) and on the volume conduction analysis of nerve action potentials as described above.

The distance (r) of the different leads of the multielectrode from the axis of the potential source, which corresponds to a given distance d was calculated as $r = \sqrt{d^2 + x^2}$, where x is the distance along the multielectrode from the lead with maximum response (Fig. 9, lower inset). Circular symmetry around the axis of the potential source is a prerequisite if the amplitude of the volume conducted spike is to be expressed as a function of the distance from the axis of the source. The fact that amplitude decreased symmetrically to both sides of the lead of the multielectrode with maximum response indicated that this prerequisite was satisfied (Fig. 7). Thus, the relation between spike amplitude and the distance x along the multielectrode could be transformed into the relation between spike amplitude and the distance r from the axis of the potential source by assuming different values of d. This transformation was applied to the three groups of motor unit spikes classified according to maximum amplitude (Table 1), and those values of d were determined for which the three logarithmic amplitude-distance relationships showed the highest degree of parallelism.

In this way maximum spike-amplitude was estimated to have been recorded at a distance of 0.085—0.15 mm from the axis of the potential source for spikes with a maximum amplitude exceeding 5 mV (curve 6, Fig. 9); at 0.14—0.18 mm for spikes of 2.5 to 5 mV (curves 5, Fig. 9), and at 0.17—0.20 mm for spikes below 2.5 mV (curve 4, Fig. 9).

It is seen that the resulting plots of the logarithm of spike amplitude as a function of the logarithm of the distance are approximately rectilinear, as was found for the isolated muscle fibre immersed in Ringer's solution. It was, however, surprising that the action potential amplitude of the isolated fibre decreased relatively less with distance than did the amplitude of the spike of the motor unit potential: the amplitude was proportional with $r^{-1.3}$ in the case of the isolated fibre (derived from HÅKANSSON 1957) and with $r^{-2.4}$ in the case of the motor unit spike.

Different maximum spike amplitudes can either originate from different distances between the recording electrode and the potential source, or they can be due to differences in the potential source with respect to radius, longitudinal spread of the action currents or the relative distribution of these currents along the source. It can be seen from curves 4, 5 and 6 of Fig. 9 that the three groups of spikes from motor unit potentials cannot derive

from identical potential sources, since there were substantial differences in amplitude at the same distance from the source. If different distances alone were responsible for the different maximum amplitudes, the three curves (4, 5, 6) would coincide. However, the average curve of spikes with low maximum amplitude (curve 4, Fig. 9) contains amplitude-distance relationships which derive from low-amplitude potential sources as well as those which derive from high-amplitude sources at greater distance.

As to be expected from the results of volume conduction analysis in nerve (LORENTE DE NÓ 1947) the duration of the positive-negative deflection of the motor unit spike increased with increasing distance from the potential source. Similarly, the duration of the positive-negative deflection of electrically evoked potentials and of the action potentials from isolated fibres increased with increasing distance. In all the duration varied relatively less with distance than did amplitude (Table 2). With an increase in distance which caused a reduction in amplitude to one tenth, the duration of the positive-negative deflection increased by 75 per cent in the case of the motor unit spike, by 100 per cent for the spike of the isolated muscle fibre in Ringer's solution (HÅKANSSON 1957) and by 75 per cent in nerve.

Number of Muscle Fibres Contributing to the Spike of the Motor Unit Potential.

According to the volume conduction analysis of the amplitude-distance relationship described in the preceding section, the maximum amplitude of high amplitude spikes (more than 5 mV) was recorded at a distance of 0.085 to 0.15 mm from the axis of the potential source. This distance represents the upper limit of the radius of the source. Areas with these radii could contain 10 to 30 muscle fibres, the mean fibre radius in the brachial biceps being 0.028 mm (BUCHTHAL, GULD and ROSENFALCK 1955 a). The high amplitude spikes originate therefore from the synchronized activity of *maximally* 10—30 muscle fibres.

As to the potential source of low amplitude spikes (maximum amplitude less than 5 mV) their amplitude was less than that of high amplitude spikes even when compared at the same distance from the potential source (curves 4 and 5, Fig. 9). In isolated frog muscle fibres action potential amplitude at any given distance is the lower the smaller the radius of the fibre (HÅKANS-

SON 1956). It is therefore reasonable to assume that motor unit spikes with low maximum amplitudes derive from sources with a smaller radius than the sources of high amplitude spikes. As to the minimum size of the potential source the estimate obtained from volume conduction does not exclude that the source may consist of a single fibre only.

Another estimate of the number of fibres contributing to the spike of the motor unit potential could be obtained from the spatial separation of different spike sources which belong to the same motor unit. With an extent of the motor unit over an area of five mm in diameter (BUCHTHAL, GULD and ROSENFALCK 1957) and with about 1,000 muscle fibres per motor unit (FEINSTEIN, LINDEGÅRD, NYMAN and WOHLFART 1955), the average distance between these fibres would be 0.16 mm, if they were homogeneously distributed within this area ($\sqrt{\frac{5}{1{,}000}} = 0.16$). In fact the spike sources were on the average 0.7 mm apart (cf. page 338), and the spike source is, therefore, estimated on the average to contain 19 muscle fibres ($n = (\frac{0.7}{0.16})^2$).

Also *histological evidence* indicates the existence of small fibre groups (subunits) which in size compare well with the number of fibres estimated to give rise to the spike of the motor unit potential. In muscles with paresis of spinal origin the muscular atrophy affects fibre groups corresponding to the loss of certain motor units. When the atrophy is mild there is a reasonable chance that the muscle fibres of each atrophic field appearing in a biopsy represent fibres from one motor unit only. WOHLFART (1949) has estimated the number of fibres in an atrophic field to 10 to 50. We have analyzed biopsies from 13 patients suffering from mild amyotrophic lateral sclerosis.[1]

A cross section of the biopsy was microphotographed and the number of fibres per atrophic field was determined on photographic magnifications. Since fixation artifacts might divide certain atrophic areas, thereby giving rise to smaller atrophic fields than actually exist, we have evaluated the maximum size of 169 atrophic fields. In this evaluation two areas were counted as distinct only when they were separated by non-atrophic muscle fibres.

The average number of muscle fibres per atrophic field was 10.2 ± 6.7 fibres. Since the degree of atrophy was slight, this

[1] Our thanks are due to ERNA CHRISTENSEN, M.D., Laboratory of Neuropathology, University of Copenhagen, for preparing the biopsy samples.

figure would correspond to the number of adjacent fibres belonging to the same motor unit. It might be such groups of fibres which give rise to the spike of the motor unit potential. In this evaluation the possible influence of peripheral sprouting (EDDS 1950, HOFFMAN 1950, WOHLFART 1955) has not been considered.

In summary, it may be concluded that both electrophysiological and histological evidence indicate that the spike of the motor unit potential derives from the synchronized discharge of a group of adjacent muscle fibres, containing maximally 30 fibres.

Discussion.

The present study of the volume conduction of the spike of the motor unit potential was performed with a multielectrode. This allowed simultaneous recording of action potentials from electrodes whose mutual localization was well defined. Displacement of a single electrode to study volume conduction in situ in our experience was endowed with serious inaccuracies, particularly when small displacements were required. The displacement of an electrode was by no means regularly correlated with a corresponding displacement relative to the active muscle fibres. Muscle fibres were often pushed sidewards and might suddenly jump back during the recording as judged from the sudden changes in the appearance of the action potentials.

We have been mainly concerned with the positive-negative deflection of the motor unit spike which has a well-defined duration of 100 to 200 μsec. Potentials evoked by local electrical stimulation at threshold intensity as well as fibrillation potentials from denervated muscle have also this positive-negative deflection of short duration. By virtue of this component they all resemble the action potential from the isolated muscle fibre immersed in Ringer's solution. This suggests that the spike of the motor unit potential derives from the discharge of one or few muscle fibres only.

LORENTE DE NÓ (1947) has quantitatively correlated the triphasic nerve action potential as led off in a volume conductor with the monophasic action potential recorded on the surface of the nerve when surrounded by an insulating medium. In this way the triphasic shape of the volume conducted nerve action potential could be accounted for. The duration of the rising phase of the monophasic potential was about the same

as that of the initial positive plus the subsequent positive-negative deflection of the triphasic response in a volume conductor. Measurements of the monophasic action potential on the surface of the potential source of the motor unit spike are not available. However, by analogy with nerve it is reasonable to assume that the short, positive-negative deflection of the motor unit spike falls within the depolarizing phase of the membrane changes as they manifest themselves *e.g.* by the intracellularly measured action potentials. The third, positive phase of the volume conducted nerve action potential which corresponds to the repolarization phase of the monophasic surface action potential was calculated to be substantially lower in amplitude than the other two phases. This asymmetry is still more pronounced for the action potential of the isolated muscle fibre immersed in Ringer's solution since a third phase only rarely can be discriminated (HÅKANSSON 1956). Fibrillation potentials and potentials evoked by local electrical stimuli at threshold intensity in situ have, however, frequently a low-amplitude third, positive phase.

From the volume conduction analysis of the amplitude-distance relationship of the spike, from the spatial separation of different spikes of the motor unit and from histological evidence the spike was estimated to originate from the synchronized activity of *maximally* thirty muscle fibres. This group of fibres is denoted "subunit" (BUCHTHAL, GULD and ROSENFALCK 1954 a). If the motor unit spike derives from more than one fibre, a duration of the positive-negative deflection of only 135 μsec (at maximum spike amplitude) requires a high degree of coincidence in the discharges of these fibres. This coincidence would require narrow limits, both of the scattering of the site of innervation and of the propagation velocities of the fibres which contribute to the spikes. With a mean propagation velocity of 4.7 m per sec a temporal dispersion of less than 135 μsec would correspond to a spatial dispersion of the site of innervation of less than 0.63 mm. This degree of scattering of motor end plates is within the anatomically probable range for the adjacent fibres of a motor unit (COËRS 1953). As to propagation velocity, however, a temporal dispersion of 135 μsec would arise from a difference in velocity of only 0.12 to 0.20 m per sec with a distance of 15 to 25 mm between site of innervation and recording electrode. Although propagation velocity over different fibres in situ only varied over a small range (BUCHTHAL, GULD and ROSENFALCK

1955 a, b), a variation of less than 0.16 m per sec seems unlikely. Therefore, a mechanism of mutual interaction between neighbouring fibres must be assumed which equalizes the propagation of their action potentials. A similar mechanism has been demonstrated for frog's nerve fibres (KATZ and SCHMITT 1940).

Our previous study indicated the influence of the high impedance of inactive fibres on the velocity of impulse propagation over muscle fibres in situ (BUCHTHAL, GULD and ROSENFALCK 1955 b). The high impedance in situ and the fact that conductivity in the longitudinal direction of the muscle is larger than in its transverse direction can affect also spike amplitudes and volume conduction. With a recording distance of about 100 μ from the axis of the potential source the highest amplitudes of motor unit spikes were five times those of the action potentials from the isolated frog muscle fibre in Ringer's solution (10 mV as compared with 2 mV). There is no difference in the intracellularly recorded membrane and action potentials of amphibian and mammalian muscle fibres (BENNETT, WARE, DUNN and MCINTYRE 1953, TRAUTWEIN, ZINK and KAYSER 1953). The higher spike amplitude in situ can originate from the higher impedance of the surrounding medium as compared with that of the Ringer's solution surrounding the isolated fibre or from a larger radius of the potential source in the case of the motor unit spike or from both. As to the relationship between spike amplitude and distance, it applied both to the action potential of the isolated muscle fibre in Ringer's solution (HÅKANSSON 1957) and to the spike of the motor unit potential that the logarithm of amplitude decreased approximately rectilinearly with the logarithm of the distance from the axis of the potential source. Within 0.75 mm the motor unit spike was reduced from several millivolts to about 100 μV (Fig. 9). A similar decrease was calculated for the action potential of nerve in a volume conductor (LORENTE DE NÓ 1947). However, the amplitude-distance relationship for the volume conducted nerve action potential is situated at a greater distance than those for the isolated muscle fibres and motor unit spikes (Fig. 9). This is due to the larger radius of the potential source and the greater region over which the action currents extend along the surface of the source in nerve as compared with individual muscle fibres and with the source of motor unit spikes. In whole nerve (radius 0.5 mm) the positive-negative deflection of the volume conducted action

potential was calculated to spread over 5.7 mm along the nerve surface (LORENTE DE NÓ 1947). In isolated frog muscle fibres (radius about 50 μ) the spread was 0.5 mm (HÅKANSSON 1957) and for the motor unit spike it averaged 0.63 mm. The logarithmic amplitude-distance relationship was steeper in the case of the motor unit spike than for the spike potentials from whole nerve and isolated muscle fibres (Fig. 9). Assuming a similar monophasic action potential for these potential sources, the difference in slope could arise either from the higher impedance surrounding the active fibres in situ or from the asymmetry in impedance or from both; the conductivity being larger along the fibres than transversely to them (BUCHTHAL, GULD and ROSENFALCK 1955 b). The logarithmic amplitude-distance relationship of the volume conducted action potentials of single spinal ganglionic cells is rectilinear over a large range as well (curve 0, Fig. 9, constructed from SVAETICHIN 1951, cell radius 30 μ). Its slope is even steeper than that for the motor unit spikes, the action potential disappearing within the noise level of the amplifier at a distance of 100 μ from the centre of the cell.

Summary.

The spike of motor unit potentials was investigated in the human brachial biceps by means of a multielectrode containing twelve small leading-off surfaces distributed over a length of 2.5 mm (Fig. 3).

Within a distance of 0.38 mm the spike amplitude decreased to one tenth of its maximum value (Figs. 6, 7).

The duration of the positive-negative deflection of the spike averaged 136 μsec (36.5° C) at the lead with maximum response, increasing by 100 μsec over the distance at which amplitude had fallen to one tenth of its maximum value (Table 2).

The six times longer duration of the total motor unit potential than of its spike component is due to superposition of temporally dispersed spike potentials from more distant parts of the motor unit (Table 2).

Applying volume conduction analysis to the motor unit spikes classified in three groups according to maximum amplitude, the relation was determined between spike amplitude and distance from the *axis* of the potential source. The logarithm of spike

amplitude decreased approximately rectilinearly with the logarithm of distance from the source. Different maximum spike amplitudes were due partly to the occurrence of potential sources of different diameter, partly to different distances of the recording electrodes from the axis of the source (Fig. 9).

The volume conduction of the spike of the motor unit potential was compared with that of electrically evoked potentials, with that of the action potential of the isolated muscle fibre in Ringer's solution and with that calculated for nerve (Fig. 9).

The number of muscle fibres contributing to the spike of the motor unit potential was estimated electrophysiologically and histologically: (1) from the amplitude-distance relationships of spikes with different maximum amplitude; (2) from the spatial separation of different spikes of the same motor unit potential; (3) from the number of adjacent atrophic fibres in muscle biopsies of patients with mild amyotrophic lateral sclerosis. The motor unit spike was thereby estimated to arise from the synchronous activity of maximally 30 muscle fibres.

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Secondary Fall in Blood Pressure Following Noradrenaline Infusion in the Cat.

By

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It is frequently noted that the rise in blood pressure following single injections of noradrenaline in doses above 5—10 μg in the anaesthetized cat is followed by (1) a fall in blood pressure to subnormal levels, and (2) a temporarily diminished sensitivity to subsequent small doses of noradrenaline.

It is common experience that the sudden withdrawal of a noradrenaline infusion in man is sometimes followed by a fall in blood pressure below the pre-infusional level, and this may necessitate a prompt recommencement of the infusion, often in a higher dosage than previously given.

Although this secondary fall has been observed by several investigators there seems to be no generally accepted explanation of this phenomenon.

BLACKET, PICKERING and WILSON (1950) have studied the effect of prolonged infusion of noradrenaline in the unanaesthetized rabbit. With doses not exceeding 3 μg/kg/min. they found that the blood pressure in most animals had a tendency to fall and that, on termination of the infusion, there was an abrupt and severe drop in blood pressure, lasting for a day or more. The authors discuss various possible mechanisms for the observed fall in blood pressure and consider a release of vasodilator substances the most likely explanation of the effect. Similar observations have been made earlier with adrenaline (BAINBRIDGE and TREVAN 1917, ERLANGER and GASSER 1919 and others).

The common use of noradrenaline as a pressor drug in human therapy, frequently in large doses, made it desirable to study the conditions in which the blood pressure fell to subnormal levels after infusion of the drug. Also an attempt has been made to analyse the mechanism of this fall, especially with a view to finding means of preventing it.

Methods.

The experiments were made on cats anaesthetized with Nembutal in a dose of 35 mg per kg body weight intraperitoneally. Blood pressure was recorded from a femoral artery. Infusions were made with the aid of a mechanical slow injection device through a femoral vein. Single intravenous injections were made through the femoral vein of the other leg.

Noradrenaline was given as bitartrate of the levo-rotatory form in Tyrode solution, acidified with HCl to pH 4. All figures are presented in terms of the hydrochloride. The infusion time was usually 10 minutes and the dose range 0.2—6.6 μg per kg per min.

Results.

1. The Effect of Infusion of Various Doses of Noradrenaline on the Blood Pressure.

The blood pressure response of the animals varied considerably; the dose required to give an initial pressure rise of 30 mm Hg varied from 0.25 to 1.4 μg/kg/min., depending on the sensitivity or reactivity of the circulatory system.

A fairly regular feature of the blood pressure level during the constant noradrenaline infusion was that it declined gradually towards a value which was 45 to 80 % of the maximal rise, on an average 60 %.

In any one animal the response to repeated infusions of the same dose was usually quite constant (Fig. 1) even after 9 infusions given at 10 min. intervals.

It was also noted in several experiments that the rise of blood pressure at the beginning of the infusion showed a small notch, the cause of which was not further investigated.

2. Post-Infusional Drop in Blood Pressure.

After the end of the infusion the blood pressure generally fell rapidly to a level well below the pre-infusion value (Fig. 1). The extent of this fall did not correlate with the height of the

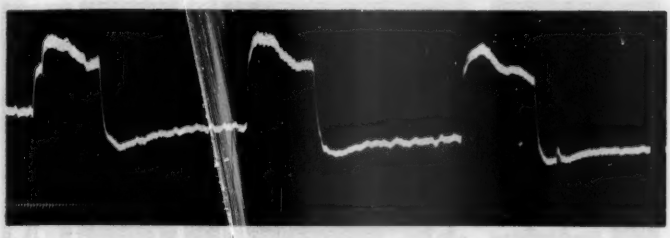

Fig. 1. Blood pressure, cat, nembutal.
Three infusions of noradrenaline, 1.0 μg/kg/min. during 10 min. each with 20 min. interval. Ordinate 60—160 mm Hg. Time 30 sec.

initial blood pressure response. Thus in 7 out of 10 cases the fall varied between 8—44 mm Hg when the blood pressure rise during the infusion was 50—66 mm Hg. On the other hand the post-infusional drop in pressure was as a rule larger when the decline in blood pressure during the noradrenaline infusion was well marked.

In 10 experiments, in which no other drugs had been given before the first infusion, the average post-infusional fall was 21 mm Hg (8—46 mm) after an average decline of pressure of 21 mm Hg (10—40) during the infusion which caused an average maximal rise of 57 (20—105) mm Hg. The two largest drops (40 and 36 mm Hg) during the infusion were followed by the two largest drops in pressure (46 and 44 mm) afterwards.

The duration of the post-infusional fall was often considerable, the initial blood pressure value not being attained for 20—40 minutes (Fig. 2). In experiment no. 2 the fall was very slight with an infusion rate of 1.9 μg/kg/min. and in experiment no. 14 a fall only appeared after the infusion rate was raised to 4 μg/kg/min.

When 10 min. infusions were repeated with 20 min. intervals (expt. 6) the blood pressure level immediately before the next infusion was lower than before the preceding one, the level falling according to the sequence 105—90—82—74 mm Hg (Fig. 1).

3. Change in Response to the Carotid Occlusion Test during and after the Infusion.

In order to obtain some information as to the reactivity of the circulatory system (Page and McCubbin 1951), regular tests

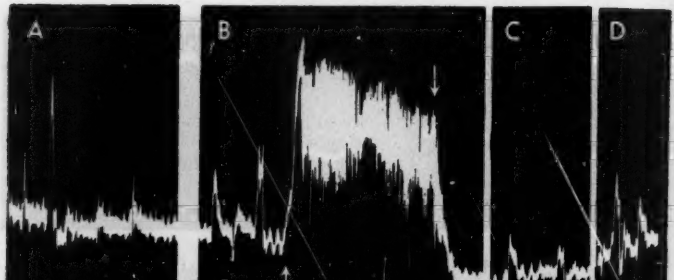

Fig. 2. Blood pressure, cat, nembutal.
A. 1, 3, 4, 5, 0.5 μg noradrenaline (NA)
2, Carotid occlusion 20 sec.
B. 6, 1 μg NA
7, carotid occlusion 20 sec.
Between arrows infusion of NA 1.5 μg/kg/min. during 10 min.
C. 6 minutes later.
8, 1 μg NA
9, carotid occlusion
D. 30 minutes later
10, carotid occlusion
11, 1 μg NA
Ordinate, 80—200 mm Hg. Time 30 sec.

were made with bilateral carotid occlusion for 20—30 seconds during and at various times after the infusion. In the majority of experiments the carotid occlusion test gave a smaller response during and immediately after the infusion, varying from 20—60 % of the original effect (Fig. 2). The carotid occlusion response then gradually increased as the secondary fall subsided. During the infusion the response was often marked by irregularities in the blood pressure level, caused by disturbances in heart rhythm.

4. Change in Response to Single Test Injections of Noradrenaline during and after Infusion.

Single intravenous doses of 0.5 μg noradrenaline were given in some experiments for the same purpose as the carotid occlusion

tests. Also in these tests the response was regularly smaller during and shortly after the infusion than before the infusion, although the decrease was less obvious than for the occlusion test (Fig. 2). It was also noted that the response to the noradrenaline test dose immediately after the occlusion test (Fig. 2) was smaller than normally.

5. Noradrenaline Infusion after Vagotomy and Bilateral Buffer Nerve Section.

Since it seemed of interest to study the influence of the homeostatic mechanisms regulating the blood pressure via the baroceptor mechanisms, infusions were also made after denervation of both carotid sinuses and bilateral vagotomy.

It was noted in some of these animals that an infusion of noradrenaline was followed by a greater secondary fall than usual. A very marked difference was noted, however, in the duration of this fall. While this ordinarily lasted 20—40 minutes, in those animals in which the buffer nerves had been sectioned, the infusion of 2 μg/kg/min. was followed by a secondary fall lasting longer than 90 minutes in some cases.

In four experiments vagotomy alone was performed. The usual increase in the carotid occlusion test response was observed, and the effect of noradrenaline infusion on the blood pressure appeared unchanged.

6. Effect of Drugs on the Secondary Fall in Blood Pressure after Noradrenaline Infusion.

a) *Calcium chloride.* It is a common observation that injections or infusions of small doses of calcium chloride tend to increase the blood pressure especially when this is low in the anaesthetized cat. Isotonic calcium chloride was therefore injected or infused in several experiments and its effect on the initial blood pressure response and the secondary fall was noted.

In a series of experiments it was noted that after injections or infusions of calcium chloride at a rate of 5—20 mg per min. the general blood pressure level rose and the secondary fall after infusion of noradrenaline was less marked. Thus in experiment 5 the blood pressure rose from a steady level of 65 mm to 95 mm during an infusion of 90 mg $CaCl_2$ over 20 minutes. The secondary fall appeared to be less marked when calcium chloride in a dose

of 2 mg/kg/min. was given concurrently with the noradrenaline infusion.

After infusion of large doses of calcium chloride, approximately 200 mg in experiment 7, the animal showed signs of regaining consciousness during the noradrenaline infusion. In experiment 3, in which 1 mg strychnin had been given and slight twitchings occurred, these became greatly accentuated during the infusion of calcium chloride. No similar effect was noted after potassium chloride.

7. Tetraethyl ammonium Bromide (TEAB).

TEAB (Etamonin, Astra) was used in 3 experiments in doses sufficient to suppress the carotid occlusion reflex (20—25 mg/kg intramuscularly). This treatment caused a typical change in the blood pressure fall after noradrenaline infusion. As seen in Fig. 3 the usually longlasting fall became of short duration after TEAB. The general blocking effect of TEAB on the autonomic transmission suggests that the longlasting part of the post-infusional fall is due to an action mediated by autonomic synapses. An additional factor must contribute to the fall however, since even after TEAB a short-lasting but constant post-infusional depression remained.

8. Ergotamine Tartrate.

This drug elicits a complex action on the circulatory system. Apart from the antisympathomimetic effect, which is of no concern in the present study since the doses were not higher than 0.2 mg per kg, ergotamine has not only a direct vasoconstrictor action on the blood vessels but also exerts a peculiar selective blocking of the part of the vasomotor centre which mediates the sympathetic activity on inhibition of the baroceptor activity, for instance by carotid occlusion (HEYMANS and REGNIERS 1929, WRIGHT 1930, EULER and SCHMITERLÖW 1944). The usual rise in blood pressure following carotid occlusion is abolished by 0.1 mg ergotamine tartrate per kg.

The short-lasting fall after noradrenaline infusion in an animal having received TEAB is completely abolished after ergotamine. This action of ergotamine can hardly be explained by the blocking of certain portions of the vasomotor centre but must in all probability be ascribed to a direct constrictor effect on the smooth

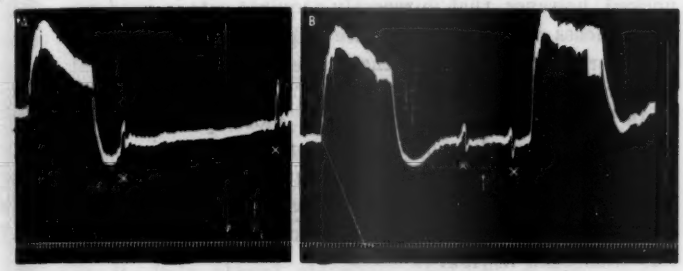

Fig. 3. Blood pressure, cat, nembutal.
Infusions of noradrenaline, 1.4 μg/kg/min. during 10 min. Between A and B 20 mg/kg TEAB i.m. At arrow 0.1 mg/kg ergotamine tartrate i.m. Crosses, carotid occlusion 30 sec.
Note slope of pressure curve after end of perfusion.
Ordinate 50—170 mm Hg. Time 30 sec.

muscle of the vascular wall (Fig. 3) preventing the initial drop of pressure.

Discussion.

The fall in the blood pressure ensuing after infusion of noradrenaline in the cat apparently consists of at least two components, one short-lasting and one of long duration. Our experimental data suggest that the fall in blood pressure after infusion of noradrenaline is due to a partial blocking action on synapses in the vasomotor system, causing a reduced vasomotor tone. This interpretation is based on the following observations: 1) after buffer nerve section, when no counter-regulation is effective on the blood pressure, the post-infusional fall is enhanced and prolonged, 2) after administration of a ganglionic blocking agent no further block would be effective and the fall is reduced to a short-lasting fraction. The noradrenaline block cannot be complete, however, since the homeostatic action due to the baroceptor mechanisms is still operating as shown by the prolongation of the effect after sectioning the buffer nerves.

Ganglionic blocking effects of noradrenaline were first reported by KONZETT (1950) and subsequently confirmed by LUNDBERG (1952) and others. This effect of noradrenaline apparently is rather weaker than that of adrenaline. From the present experiments, however, it seems to cause strong and lasting effects when administered by infusion in not too small doses. We have

noticed, however, that in recently acquired cats in good condition larger doses of noradrenaline are required to cause a significant secondary fall than in cats that have stayed in the animal house for some time.

As to the post-infusional fall in blood pressure which still appears after ganglionic blocking agents its origin is still obscure. Among possible causes, metabolites of noradrenaline having a direct vasodilator action on the vessels might be considered. A duration of some 5 minutes of this action would not be incompatible with such a view.

If the explanation of the fall in blood pressure occurring after infusions of noradrenaline is correct, it might be possible to reduce the effect by administration of deblocking agents. Preliminary experiments with eserine in small doses seem to support the assumption of a transmission block since this drug reduced or abolished the secondary fall in the atropinized animal.

Summary.

The rise in blood pressure caused by an infusion of noradrenaline in the cat is frequently followed by a marked fall in blood pressure to subnormal levels, often lasting for 20—40 minutes.

The sensitivity to single injections of noradrenaline is diminished during the first part of this period. After section of the buffer nerves the fall after noradrenaline infusion is prolonged and often enhanced.

Intravenous infusions of calcium chloride reduce the blood pressure fall after noradrenaline.

Only a brief and moderate fall is seen in animals having received TEAB in transmission blocking doses. This fall can be abolished by small doses of ergotamine.

It is concluded that the fall in blood pressure after noradrenaline is partly due to a blocking effect on transmission within the vasomotor system.

Certain implications of the present observations for the therapeutic use of noradrenaline infusions in man are briefly discussed.

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Effects of Certain Biologically Occurring Substances on the Isolated Intestine of Fish.

By

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While the response of smooth muscle organs to a variety of biologically occurring substances has been extensively studied in mammals, few investigations have been carried out on fish. Since information about the relative activity of various hormones and autonomic drugs not only may aid in the elucidation of the receptor mechanisms of smooth muscle but also may serve as a means of pharmacological characterization of various species, some studies have been performed with this aim on the intestines of representatives of various classes of fish.

Interesting differences in the response of the intestine of elasmobranchs and teleosts to adrenaline have been observed. Thus adrenaline was found to stimulate the intestine of elasmobranchs (DREYER 1928, NICHOLLS 1933), but to inhibit it in teleosts (FÄNGE 1948, DREYER 1949).

While acetylcholine stimulates the intestinal motility in both elasmobranchs and teleosts this action is said to be abolished by atropine in the latter group only (DREYER 1949) suggesting that the mode of action is different in this species. However, NICHOLLS (1933) in a detailed study found that the action of acetylcholine on the elasmobranch intestine was abolished by atropine as are muscarine-like actions generally.

Methods.

Different parts of the intestine of freshly caught specimens of teleosts (*Pleuronectes platessa, Labrus berggylta, Gadus callarias, Lophius*

piscatorius, *Anguilla vulgaris*), elasmobranchs (*Squalus acanthias* and *Raja batis*), and a cyclostome (*Myxine glutinosa*) were removed and suspended in a 75 ml bath. In some cases the whole length of intestine was used while in larger specimens of fish either the proximal or the distal part was used. As a rule no differences were noted in the response to various substances between the two parts. The immersion fluid consisted of 1 part of deep-sea water mixed with 1—1.5 parts distilled water at 18—25° C, aerated with air. In experiments with intestine of elasmobranchs the following solution was used: 3 l deep sea water, 2 l dist. water and 150 g urea. For *Myxine* 2/3 deep sea water was used. The responses to the following drugs were studied:

Acetyl choline hydrochloride	(ACh)
Histamine dihydrochloride	(Hi)
5-Hydroxytryptamine creatinine sulphate	(5-HT)
Noradrenaline hydrochloride	(NA)
Adrenaline hydrochloride	(A)
Substance P	(P)

All figures are given in terms of the bases. The salts were dissolved in slightly acidified distilled water which in itself had no action in the amounts given. The amounts of the different substances have been expressed as concentrations (μg per ml) throughout.

Results.

Acetylcholine.

Acetylcholine (ACh) caused marked contraction in all intestinal preparations studied, in concentrations of about 0.05 μg/ml. In *Pleuronectes* and *Lophius*, which showed the highest sensitivity to this drug, contractions were obtained in concentrations as low as 0.02 μg/ml. The sensitivity of elasmobranch intestine to ACh was notably less than that in teleosts. Thus in intestinal preparations of *Squalus acanthias*, which reacted readily to 0.04 μg adrenaline per ml, only slight responses were found with 20 times stronger concentrations of ACh. In *Raja* the response to ACh was likewise relatively small. Usually the contractions rapidly reached a maximum, shortly followed by relaxation. Long sustained contractions were, however, observed in *Anguilla* (Fig. 3) and also in *Myxine* as previously observed by FÄNGE (1948). In a preparation of rectum from *Squalus* ACh caused a marked increase in the frequency of the spontaneous contractions.

The present experiments add further support to the common observation that ACh usually has a stimulating effect upon smooth muscle in animals. This action was always abolished,

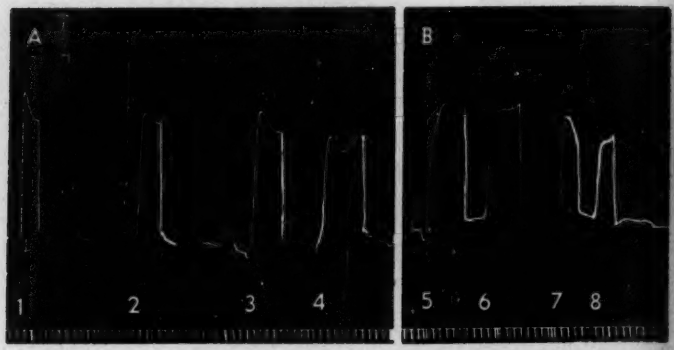

Fig. 1. *Pleuronectes platessa*. Distal intestine, 50 % deep sea water. *A* before, *B* after atropine 1 : 1 mill. Time $^1/_2$ min.

1. 0.043 μg/ml ACh.
2. 0.018 μg/ml 5-HT.
3. Substance P from cod intestine.
4. 0.65 units per ml Substance P from mammalian intestine.
5. Same as 3.
6. 1.3 units per ml Substance P.
7. 0.8 μg/ml Hi.
8. 0.012 μg/ml 5-HT.

even in elasmobranchs (cf. DREYER), after the addition of atropine sulphate 1 : 1 million to the bath fluid.

Although ACh was very active in all teleosts it was not the most active of the drugs studied in all species. Thus in *Pleuronectes* and *Labrus* 5-hydroxytryptamine (5-HT) was regularly about 2—4 times as active as ACh.

Figs. 1—6 show the effects of ACh on isolated pieces of intestine of *Pleuronectes*, *Labrus*, *Anguilla*, *Lophius*, *Raja* and *Myxine*.

Histamine.

The histamine (Hi) effects were well marked in *Pleuronectes*, *Labrus*, and *Gadus*, and consisted in a rapid and well sustained contraction. In all three species histamine was less active than ACh, however. In *Anguilla* and *Lophius* no definite effects were obtained with doses as high as 0.5—1.6 μg per ml (Figs. 3 and 4) and in *Myxine* similar amounts caused only a minimal effect (Fig. 5).

5-Hydroxytryptamine.

Strong contractions were observed with 5-HT on *Pleuronectes* and *Labrus*, in which species it was the most potent drug tested.

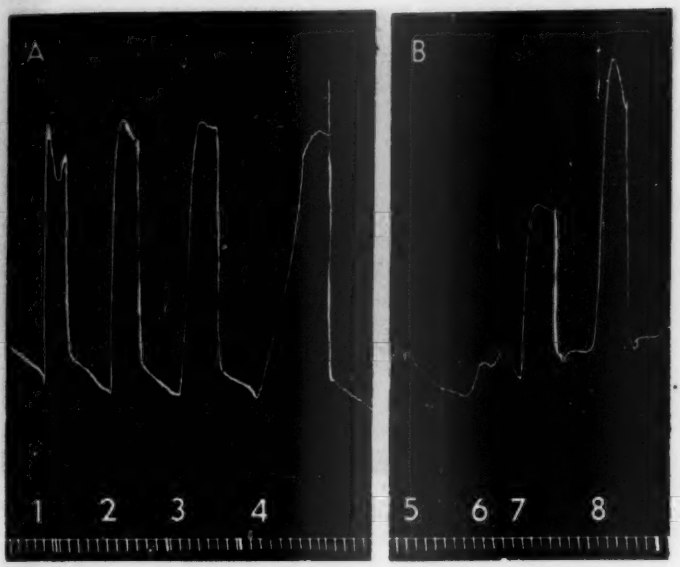

Fig. 2. *Labrus berygylta.* Distal intestine, 40 % deep sea water. *A* before, *B* after atropine 1 : 1 mill. and Antasten 1 : 200,000. Time $^1/_2$ min.

1. 0.12 μg/ml Hi.
2. 0.064 μg/ml ACh.
3. 0.023 μg/ml 5-HT.
4. Substance P from fish intestine.
5. 0.12 μg/ml Hi.
6. 0.064 μg/ml ACh.
7. Same as 4.
8. 0.023 μg/ml 5-HT.

Definite actions were found in *Pleuronectes* with concentrations as low as 0.005 μg/ml. Both in *Gadus* and in *Anguilla* the effects of 5-HT were 2—5 times less than those of ACh and in *Gadus* even weaker than those of histamine. In *Lophius* the effect of 5-HT was about 25 times smaller than that of ACh.

In *Myxine* the effect was dubious with doses up to 1 μg per ml of 5-HT.

Substance P.

This polypeptide, which strongly stimulates mammalian intestine (EULER and GADDUM 1931, PERNOW 1953), has recently been found to occur in intestine and brain of teleosts (*Gadus*) and elasmobranchs (*Squalus*), (EULER and ÖSTLUND 1956). It was tested on the intestine of *Pleuronectes*, *Labrus* and *Raja* both in purified form from mammalian intestine and as crude

Fig. 3. *Anguilla vulgaris*. Distal intestine, 50 % deep sea water. Time 1/2 min.

1. 0.13 µg/ml ACh. First arrow 0.5 µg/ml NA
   Second » 0.1 » A
2. 0.064 µg/ml ACh. First » 1 » NA
   Second » 0.05 » A
3. 0.064 µg/ml ACh. Arrow 5 » NA
4. 0.23 µg/ml 5-HT.
5. 0.6 µg/ml Hi.

extract from fish intestine (Figs. 1, 2 and 6). The action persisted after the addition of atropine 1 : 1 million and the antihistamine antasten 1 : 200,000. Since 1 unit of the purest preparation Substance P obtained by PERNOW (1953) is contained in 0.3 µg substance, the activity is comparable with that of the other drugs tested.

Adrenaline and noradrenaline.

Both substances caused inhibition of the intestinal tone in teleosts but showed quantitative differences, adrenaline (A) being considerably more active than noradrenaline (NA). This was particularly so in the eel where 1 µg NA per ml, given at the peak level of an ACh contraction, had no effect, while 0.05 µg A per ml caused a well marked inhibition. Only after raising the dose of noradrenaline to 5 µg per ml was a similar inhibition observed (Fig. 3). In *Myxine* FÄNGE (1948) found inhibition of tone and rhythmic activity in doses of 1—10 µg per ml of adrenaline.

The finding of DREYER (1928) and NICHOLLS (1933) that adrenaline stimulates the smooth musle of the digestive tracts in elasmobranchs has been confirmed. In addition we have shown

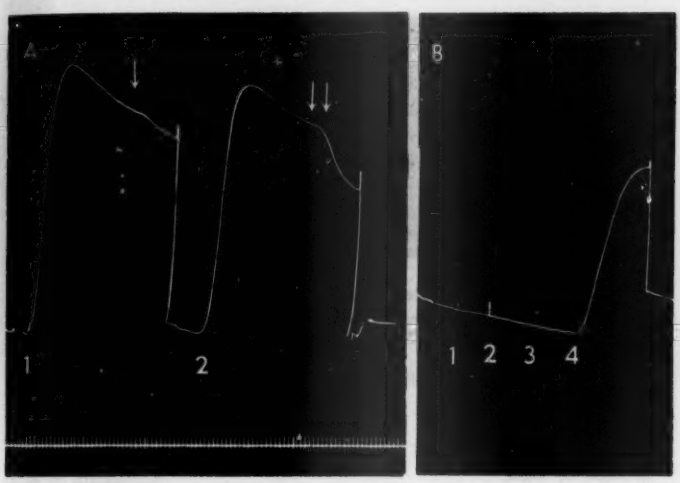

Fig. 4. *Lophius piscatorius.* Distal intestine, 50 % deep sea water. Time 10 sec.
A1 and A2. 0.025 μg/ml ACh., at arrow 0.13 μg/ml NA, at double arrow 0.026 μg/ml A.
B1. 1.6 μg/ml Hi.
B2. Atropine 1 : 1 mill.
B3. 0.025 μg/ml ACh.
B4. 0.3 μg/ml 5-HT.

that the stimulant action of noradrenaline is much less than that of adrenaline (Fig. 6). In order to obtain a similar contraction as with adrenaline on the intestine of *Squalus* a 30 times higher dose of noradrenaline was required. It was also noted that adrenaline in about 0.5 μg/ml caused relaxation of the intestine of *Raja* during contraction induced by ACh (Fig. 6 A).

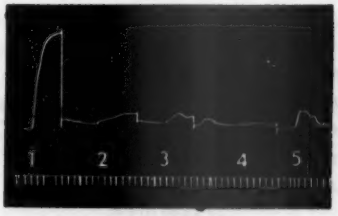

Fig. 5. *Myxine glutinosa.* Distal intestine, 2/3 deep sea water. Time ½ min.
1. 0.34 μg/ml ACh.
2. 0.24 μg/ml 5-HT.
3. 0.64 μg/ml Hi.
4. 0.48 μg/ml 5-HT.
5. 1.3 μg/ml Hi.

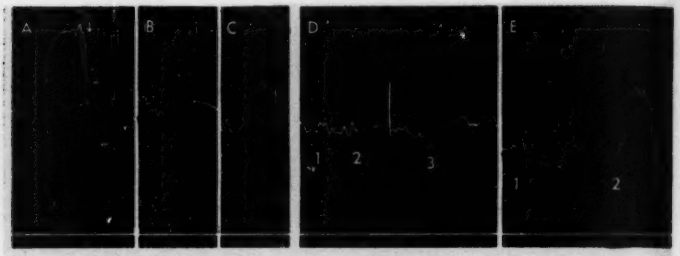

Fig. 6. *Raja batis.* Whole intestine, 50 % deep sea water. Time 10 sec.
A. 0.42 µg/ml ACh, at arrow 1.3 µg/ml A.
B. 1.3 unit/ml Substance P.
C. 0.80 µg/ml Hi.
Between C and D atropine 1 : 500,000.
D1. 2.5 µg/ml ACh.
D2. 1.3 unit/ml Substance P.
D3. 0.6 µg/ml 5-HT.
E1. 1.3 µg/ml NA.
E2. 1.3 µg/ml A.

Relative efficiency of drugs stimulating intestinal activity in different groups of fish.

As mentioned above certain differences in the relative activity of the drugs tested were noted in the five teleosts, the two elasmobranchs and in *Myxine*. Thus 5-HT was the most active drug in *Pleuronectes*, *Labrus* and the proximal part of the intestine in *Anguilla* while ACh was the most active in *Lophius*, *Gadus*, in the distal part of the intestine of *Anguilla* and in *Raja* and *Myxine*.

For comparison it may be mentioned that no response to any drug was obtained with a preparation of the intestine of the crustacean *Nephrops norwegicus*.

The action of the drugs on the different fish groups and species is shown in Table I.

Discussion.

In several species of fish a surprisingly high degree of sensitivity was observed for some biologically occurring substances. Particularly noteworthy was the strong action of 5-HT in *Pleuronectes* and *Labrus*, suggesting the possibility of 5-HT as a biologically important substance for the intestinal motility in certain teleosts.

The quantitative difference in activity between adrenaline and noradrenaline, which is often noted in mammals, is well marked in the experiments on the *Anguilla*, *Lophius*, *Raja* and *Squalus*,

Table I.

Effects of various biologically occurring ergones on the intestinal activity of fish.

Class Species	Acetyl-choline	Adr.	Noradr.	5-HT	Hist.
Cyclostome					
Myxine	+	—	(—)	0	(+)
Elasmobranchs					
Squalus	(+)	++	+	(+)	
Raja	+	+	(+)	(+)	(+)
Teleosts					
Pleuronectes	++			+++	+
Labrus	++			+++	+
Gadus	++			+	+
Lophius	+++	— —	(—)	++	0
Anguilla	++	— —	(—)	++	0

+ = contraction
— = relaxation
0 = no action
= not tested

in which equivalent effects required a dose of NA some 30—100 times greater than A. This effect was inhibitory in teleosts and stimulatory in elasmobranchs in confirmation of earlier observations.

Substance P exerted a strong and sustained contraction in all intestinal preparations tested. This is of particular interest since Substance P occurs in the intestine both of teleosts and elasmobranchs. Further studies are in progress regarding the occurrence of Substance P in more primitive marine animals.

The response in different parts of the intestine has not been systematically studied. However, in most species no difference was found in the reactivity of the proximal and the distal parts of the intestine. Only in *Anguilla* was a difference in the relative sensitivity to acetylcholine and 5-HT noted between the proximal and distal parts.

Summary.

5-Hydroxytryptamine strongly stimulated intestinal motility in *Pleuronectes* and *Labrus*. It was less active in *Anguilla*, *Lophius*, *Squalus* and *Raja*, and had little or no action in *Myxine*.

Acetylcholine stimulated the isolated intestine in all species tested, although this effect was small in *Squalus*.

Histamine contracted the intestine in *Pleuronectes*, *Labrus* and *Gadus* but had either a small or no action in *Lophius*, *Anguilla*, *Raja* and *Myxine*.

Noradrenaline had only 1/30—1/100 of the inhibitory action of adrenaline on the intestine of *Anguilla* and *Lophius*, and 1/30 of the stimulating action of adrenaline on the intestine of *Squalus*.

Substance P stimulated the intestine of all species tested.

We are indebted to Dr. G. GUSTAFSON and the Staff of the Kristineberg Marine Biological Station for their kind co-operation in this work.

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Unsaturated Fatty Acid Composition of Subcutaneous Fat in Rats in Relation to Diet.

By

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The aim of the present experiment is to follow the variation in the unsaturated fatty acid content of the subcutaneous fat depots of rats reared on 1) a nearly fat-free diet, and 2) a diet contaning 28 % hydrogenated peanut oil as shown in table 1.

Experimental.

Three male rats, nos. 1, 2 and 3, of initial weights 33, 43, and 46 grams, respectively, about 3 weeks old were placed on the fat-free diet the composition of which is shown in table 1.

Three other male rats, nos. 4, 5, and 6, of initial weights 30, 40, and 43 grams, respectively, were placed on the 28 % hydrogenated peanut oil diet (table 1). After 98 days on the latter diet when the animals were in a very poor condition they were shifted to the fat-free diet supplemented with 16 mg linoleic acid per animal per day. During the experimental period samples of subcutaneous fat tissue were taken weekly by the following procedure: The rat was anesthetized with ether, a 3 mm incision was made in the inguinal region (right and left side alternatively). By means of a pair of tweezers a part of the fat tissue was drawn out through the incision, and a suitable amount (about 100 mg) was cut off. The main portion of the fat tissue was put back

and the incision was closed with catgut.[1] The procedure is very simple and takes about 5 minutes per animal.

The fat was isolated from the samples of fat tissue by extraction with light petroleum after grinding the tissue in a mortar with sodium sulfate. The light petroleum was evaporated in a stream of oxygen-free nitrogen, whereafter the fat was analyzed for dienoic acid, total and preformed conjugated, according to the procedure of HAMMOND and LUNDBERG (1953), (Sample size 5—10 mg, isomerization time 15 minutes.) The difference between total and preformed conjugated dienoic acid was considered to represent linoleic acid.

Results and Discussion.

The results are presented in figs. 1 and 2. Each figure shows the weight of the animal, the percentage of linoleic acid, and of conjugated dienoic acid in the isolated fat plotted against time. On fat-free diets as well as on diets with 28 % hydrogenated peanut oil the content of linoleic acid decreases rapidly during the first 30 days of experiment.[2] At the same time the growth curve is nearly a straight line. Thereafter the percentage of linoleic acid remains almost constant at a certain level or declines slightly.

The fat tissue of rat no. 1 was depleted faster than that of rat nos. 2 and 3 with respect to linoleic acid (fig. 1).

This is in accordance with the shorter time required for development of scaly tail in this rat: Rat no. 1 was scaly all over the tail (degree 4[3]) (figs. 3 A and 3 B) after 50 days on the fat-free diet, while it took 80 days for rat no. 2 to reach the same degree of tail symptom. Rat no. 3 never developed a scaliness of degree 4 during the experimental period.

The slower growth of rats receiving hydrogenated fat (fig. 2 compared with fig. 1) has been described earlier by DEUEL, GREENBERG, ANISFELD and MELNICK (1951), AAES-JØRGENSEN (1954) and others.

The content of *conjugated* dienoic acid in the subcutaneous fat

[1] In two instances the samples of depot fat were taken from other sites than the inguinal region: The 78th day of experiment samples were taken from the region of Vena jugularis, and the 131st day from the region near the testes.

[2] In the left inguinal region of rat no. 6 an abscess was withdrawn on the 47th day of experiment; the presence of the abscess may explain the increased percentage of linoleic acid in the sample of subcutaneous fat taken from the same place on the same day. The high amount of linoleic acid found on the 107th day of experiment may have a similar cause, although in this case an abscess has not been recorded.

[3] Degrees 1, 2, 3 and 4 correspond to mild, moderate, marked, and very marked scaliness of the tail, respectively.

Table 1.

	Fat-free diet %	Hydrogenated peanut oil containing diet %
Hydrogenated peanut oil	0	28
Sucrose	74	46
Vitamin Test Casein[1]	20	20
Salt mixture[2]	5	5
Vitamin mixture[3]	0.5	0.5
Choline chloride	0.5	0.5

Vitamins A and D_3 were given in the form of an aqueous solution with Tween 80. 0.1 ml of this solution was administered three times weekly by means of a precision syringe, whereby each rat was furnished with an average amount of 17 i. u. A and 2.6 i. u. D_3 per day. A small amount of soybean oil present in the solution supplied an average amount of 1.3 μg linoleic acid per rat per day. This amount is considered too small to have influenced the depletion of the rats with respect to this fatty acid.

[1] From Genatosan Ltd., Loughborough, England. (Precipitated from skim milk with acetic acid, washed successively with water, alcohol, and ether, and dried.)

[2] McCollum & Simmonds' (1918) salt mixture no. 185, supplemented with 13.5 mg KI, 139 mg $CuSO_4$, $5H_2O$, and 556 mg $MnSO_4$, $4H_2O$ per 100 g.

[3] 0.5 g of the mixture consisted of: biotin 0.05 mg, folic acid 0.05 mg, p-aminobenzoic acid 35 mg, thiamine hydrochloride 5 mg, riboflavin 5 mg, pyridoxin hydrochloride 5 mg, calcium pantothenate 5 mg, nicotinic acid 8 mg, inositol 15 mg, ascorbic acid 5 mg, DL-α-tocopheryl acetate (Ephynal, Roche Products Ltd.) 5 mg, dicalcium salt of 2-methyl-1,4-naphthohydroquinone diphosphoric acid ester (Synkavit, Roche Products Ltd.) 1 mg, and sucrose to 500 mg.

of rats on the fat-free diet (fig. 1) decreased slightly during the first 20 to 25 days to a level of 0.24—0.25 %, while that of the rats fed 28 % hydrogenated peanut oil (fig. 2) increased to 2.2—2.3 %. The latter value remained almost constant as long as the diet contained hydrogenated oil. This high value is surprising in view of the fact that the hydrogenated oil fed contained only 0.1 % of *conjugated* dienoic acid. Unpublished data from our laboratory have shown that if rats are fed 28 % *non*-hydrogenated peanut oil no accumulation of conjugated dienoic acid occurs in the fat tissue in spite of the fact that the non-hydrogenated oil contains more *conjugated* dienoic acid (about 0.2 %) than the hydrogenated. This observation is suggestive of a competition between linoleic and conjugated dienoic acids.

Holman (1951) found alkali-*conjugated* linoleic acid to be toxic to depleted rats when fed in doses of 40 mg per rat per day. Therefore, our finding of a marked depression of growth beginning at the time when the conjugated dienoic acid attains its maximal

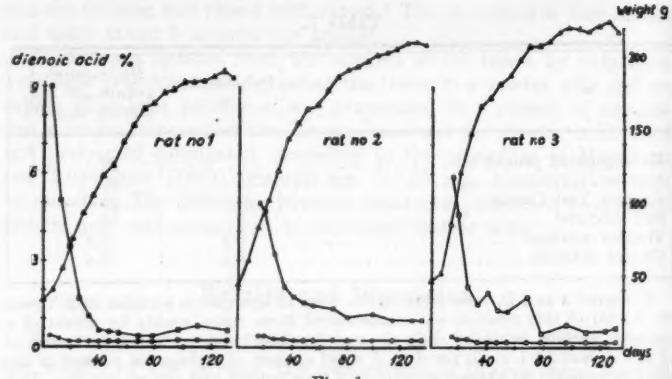

Fig. 1.

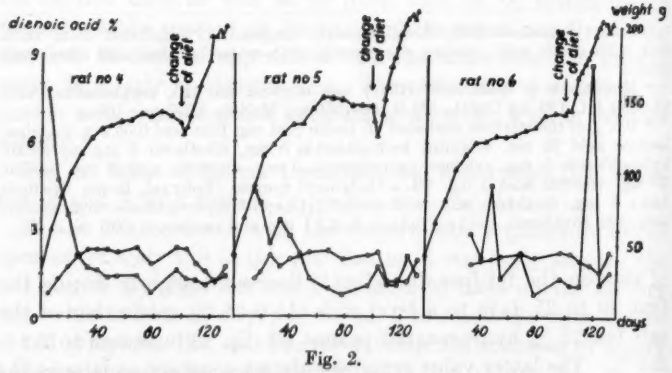

Fig. 2.

Figs. 1 and 2 represent weight in grams (—△—△—△—), linoleic acid (—□—□—□—), and conjugated dienoic acid (—○—○—○—) in per cent of depot fat of rats nos. 1—6 during the experimental period of 131 days from the time of weaning. The diet of rats nos. 1, 2, and 3 (fig. 1) was fat-free; that of rats nos. 4, 5, and 6 (fig. 2) contained 28 % hydrogenated peanut oil. The diet of rats nos. 4, 5, and 6 was changed after 98 days as described in the text

value and dienoic acid is at its minimum value could be interpreted as an unfavorable effect of the *conjugated* acid. It must be borne in mind, however, that EVANS and LEPKOVSKY (1932) found a growth depressing effect of glyceryl-laurate which is not supposed to contain conjugated dienoic acid. Therefore, retarded growth might as well be due to the presence of high dietary fat without essential fatty acids.

If, in fig. 2, extrapolation is permissible it lasted about 40 days

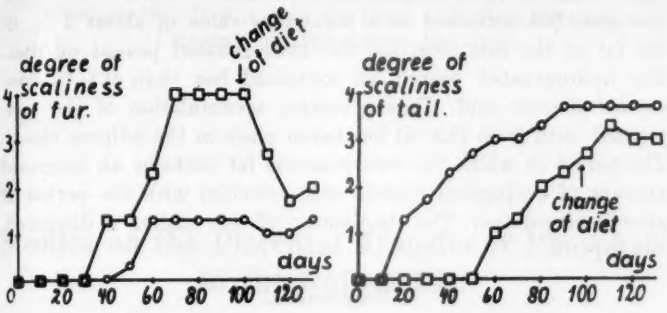

Fig. 3 A. Fig. 3 B.

Fig. 3. Average degree of scaliness of fur (A) and tail (B) during the experiment.
—○—○— Average for rats nos. 1, 2, and 3 (fat-free diets).
—□—□— Average for rats nos. 4, 5, and 6 (diets with 28 % hydrogenated peanut oil).
Degrees 1, 2, 3, and 4 correspond to mild, moderate, marked, and very marked scaliness, respectively.

after exclusion of the hydrogenated oil from the diet before rat no. 4 was depleted of the conjugated dienoic acid which had accumulated in its subcutaneous fat.

The development of scaliness of skin and tail as described by BURR and BURR (1929) is shown in fig. 3 A and B. The values plotted are averages for three animals, the scaliness of which is scored as mentioned above. Rats nos. 4, 5, and 6 fed 28 % hydrogenated peanut oil developed scaly skin earlier and to a higher degree than rats nos. 1, 2, and 3 (fat-free diets). Rats nos. 1, 2, and 3 developed scaly tail earlier and to a higher degree than rats nos. 4, 5, and 6.

The rats reared on the fat-free diet and on the diet with hydrogenated peanut oil showed the histological changes of the testes previously described by AAES-JØRGENSEN, FUNCH, ENGEL and DAM (1956).

Summary.

The subcutaneous fat from three male rats reared on a fat-free diet and three other male rats reared on a diet containing 28 % hydrogenated peanut oil was analyzed at regular intervals for linoleic acid and *conjugated* dienoic acid. The content of linoleic acid declined rapidly to a low level in both groups. The level of *conjugated* dienoic acid was almost constant in rats on the fat-

free diet, but increased to a maximum value of about 2 % in the fat of the rats receiving the hydrogenated peanut oil diet. The hydrogenated peanut oil contained less than 0.1 % *conjugated* dienoic acid. Thus a certain accumulation of the conjugated acid from this oil has taken place in the adipose tissue. The period in which the subcutaneous fat contains an increased amount of conjugated dienoic acid coincides with the period of growth retardation. The significance of this finding is discussed.

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Studies on the Placental Transfer of Phosphate in the Guinea Pig.

I. The Transfer from Mother to Foetus.

By

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FLEXNER and his co-workers were the first to use isotopes in placental permeability studies (FLEXNER and ROBERTS 1939). By this method substances normally present in the organism could be studied without changing their physiological, delicately balanced concentrations. Thereby it became possible to study the transfer to the foetus of various inorganic constitutents about which very little had been known.

WILDE, COWIE and FLEXNER (1946) studied the transfer of phosphate to the guinea pig foetus and found that the relation between the amount transferred and the amount retained in foetal growth during the same period of time, a ratio which they called the "safety factor", was unity or even less, while the corresponding ratios for sodium and water were of the order of 50 and 500, respectively. They did not take the placental uptake of radiophosphate into account, but in a later study of the placental transfer of iron (VOSBURGH and FLEXNER 1950), the necessity was demonstrated of considering also the placental uptake of isotope in studies of substances moving relatively slowly across the placental barrier.

WILDE, COWIE and FLEXNER (1946) considered their results with inorganic phosphate to be in agreement with a transfer by diffusion. However, in man and all other species so far studied

(cow, sheep, horse), the concentration of inorganic phosphate has been found to be higher in the foetal than in the maternal plasma (NEEDHAM 1931). WILDE, COWIE and FLEXNER did not determine the phosphate concentration in the foetal plasma of the guinea pig. If also in this species it is higher than the maternal concentration, diffusion is unlikely to account for the transfer across the placenta. A more detailed study of the passage of phosphate between mother and foetus in the guinea pig has therefore been undertaken, in the hope of gaining some information as to the mechanism of transfer of phosphate to the foetus.

Theoretical Considerations.

The fundamental assumption for the use of isotopes in permeability studies is that the exogenous isotope behaves in the organism exactly in the same way as the endogenous isotope of the same element. For the calculation of the transferred amount of inorganic phosphate the equation (1) has been used:

$$\frac{^{32}P_m}{P_m} = \frac{^{32}P_{pl}}{P_{pl}} = \frac{^{32}P_f}{P_f} \qquad (1)$$

in which $^{32}P_m$ denotes the concentration of the exogenous, radioactive phosphate and P_m the concentration of inorganic phosphate in the plasma on the maternal side of the placental barrier, whereas $^{32}P_{pl}$ is the amount of radioactive phosphate and P_{pl} the endogenous phosphate taken up by the placenta, and $^{32}P_f$ and P_f the corresponding amounts transferred to the foetus within the time of experiment.

The equation (1) is only valid if there is no return of radiophosphate from the foetus or placenta to the mother within the time of experiment. WILDE, COWIE and FLEXNER (1946) considered the return from the foetus to be negligible as long as the foetal uptake was linear with time, a condition they found to be satisfied up to one hour after injection of the mother. From the placenta, on the other hand, there might be a considerable return to the mother even within this period, and this has to be taken into account in the evaluation of the results, as will be pointed out in the discussion.

If the plasma of the pregnant guinea pig, as previous studies indicate (FUCHS and FUCHS 1954 a, b, c), contains a labile, nondiffusible phosphorus compound which during the procedure for phosphorus determination is broken down to inorganic phosphate

and determined as such, the value obtained for P_m is too high. In order to investigate whether this has any influence on the results, the transfer across the placenta was studied both after administration of radioactive phosphorus as inorganic phosphate and of donor plasma, labelled in vivo with ^{32}P for one hour to bring the labile fraction into equilibrium with the inorganic phosphate with regard to radioactivity (FUCHS and FUCHS 1954 a).

As the concentration of ^{32}P in the maternal plasma is not constant during the experiment, but is rapidly decreasing, it is necessary to determine the average concentration during the experiment and to use this as an expression for the $^{32}P_m$. For this purpose it is necessary to establish the disappearance curve of ^{32}P in the maternal plasma after intravenous injection. To avoid multiple blood sampling in the experimental animals, the curve was established beforehand on the basis of preliminary experiments, mainly on non-pregnant animals, including those in which the renal excretion was studied (FUCHS and FUCHS 1954 a). It was shown that the disappearance curve after administration of ^{32}P as inorganic phosphate had a steeper slope than the one after injection of labelled donor plasma, but the two curves approached each other after one hour. By establishing the disappearance curves beforehand it was possible to determine the average plasma concentration with only one or two blood samples from the experimental animals. The initial concentration of ^{32}P was calculated by dividing the injected amount by the plasma volume. For the latter the value 3.9 ml per 100 g "maternal weight", *i. e.*, the total weight of the pregnant animal minus the weight of the foetuses, was used (FUCHS 1953).

The factors $^{32}P_f$ and $^{32}P_{pl}$ were determined by measuring the total amounts of ^{32}P in the foetus and placenta respectively, at the end of the experiment.

Procedure.

The animals were anaesthetized with Nembutal intraperitoneally, a dose of 30 mg per kg body weight being usually sufficient to give a satisfactory anaesthesia for half an hour or more.

Labelled donor plasma was obtained from non-pregnant guinea pigs. A small amount of a highly active solution of ^{32}P as inorganic phosphate was injected into a vein on the foreleg of the anaesthetized animal. After one hour blood was withdrawn from the exposed carotid artery with chilled, heparinized syringes and immediately centrifuged. The

injected activity was chosen so that one ml of donor plasma would have the same activity after one hour as was injected directly to those experimental animals which received radioactive inorganic phosphate. This was obtained by giving the donor animals about a thousand times as much activity as was used in the experimental animals.

As the first step, one of the carotid arteries was exposed on the pregnant animals. The cranial end was tied and a steel cannula with a stylet was inserted in the caudal end with a clamp applied to the artery below the tip of the cannula.

The intravenous injection of the radioactive solution or donor plasma was made in a subcutaneous vein which was found through a small incision in the foreleg. The needle was kept in the vein for a little while to make sure that all the injected fluid was removed by the blood stream, and after its withdrawal the bleeding was stopped with Gel-Foam. Injection into such small, fragile and easily collapsible veins is rather difficult, and in a few cases the animal had to be discarded on account of paravenous injection. Even with the needle definitely in the vein it is difficult to ascertain that all the injected fluid is brought into circulation. This constitutes a definite source of error, but the determination of the maternal plasma activity a few minutes after injection and at the end of the experiment provided a certain control.

The removal of the foetuses and placentas through an inferior median laparotomy was made after an interval varying from 15 to 50 minutes, but in the majority of the cases after about half an hour. One of the uterine horns was brought out and opened by a transverse incision, avoiding the uterine vessels as much as possible. The membranes were ruptured and the first foetus was delivered with division of the umbilical cord between clamps. The placenta and the attached subplacenta were then removed from the uterus, separated from the membranes and put into a beaker for weighing and ashing. The next foetus was then delivered, and so on. Only in a few instances were there any longer intervals between the delivery of the individual foetuses. The foetuses were killed with a blow on the head, wiped off, measured and weighed, and put into beakers for drying and ashing. Large foetuses were cut into pieces to facilitate drying, carefully avoiding loss of blood. Finally the thorax of the mother was opened and a large blood sample withdrawn from the heart, upon which the animal was sacrificed.

Chemical and Radiochemical Determinations.

All blood samples were collected in chilled syringes and transferred to tubes containing small amounts of dried, phosphorus-free heparin. They were centrifuged and separated as soon as possible and kept cold until phosphate determinations could be carried out. Deproteinisation was done with ice-cold 10 % trichloracetic acid.

The foetuses and placentas were dried overnight at 110° C. and ashed in a muffle at 400—450° C. If ashing was not complete, some nitric acid was added and the ashing repeated. The ash was dissolved and hydrolyzed in dilute hydrochloric acid and made up to a certain volume,

from which aliquots were taken for phosphate and radiochemical determinations.

In those instances in which blood samples were ashed for total phosphorus determinations, calcium chloride was added before ashing to prevent the formation of free phosphoric acid which would evaporate at the high temperature.

Phosphorus determinations were carried out by the method of FISKE and SUBBAROW (1925), as modified by LEPAGE (1949). Later in the course of this study the modification described by GOMORI (1942) was adopted. Double determinations were carried out in almost all instances, and from a series of samples of known phosphate concentration the accuracy of the determination was found to be of the order of one microgram per ml.

Determination of radioactive phosphorus was carried out as described by LEVI (1941) by precipitation of the phosphorus from an ammoniacal solution as magnesium ammonium phosphate. Inactive phosphate was added to samples of low phosphorus content to give precipitates of approximally equal weight. Radioactivity was measured with a bell-shaped GEIGER-MÜLLER tube with a mica end-window, connected to a Victoreen amplifier or a Tracerlab autoscaler. The precipitations were always carried out in duplicate, and only in rare instances were there signs of loss of radioactive phosphate during filtration.

The gestation age was calculated from foetal weight and length and the litter size with the aid of the tables of DRAPER (1920) and IBSEN (1928).

Results.

Inorganic Phosphate in the Maternal and Foetal Plasma.

The concentration of inorganic phosphate was in all cases considerably higher in the foetal than in the maternal plasma. In 32 guinea pigs between the 31st day of gestation and term the concentration varied between 30 and 60 μg P per ml with an average of 45 μg P/ml, or about the same as in non-pregnant animals. No correlation with gestation age was found. In an animal 29 days pregnant the exceptionally high value of 67 was found, but since no other observations were made before the 30th day, it is not known whether the concentration is generally higher in the first part of gestation.

In 30 foetuses between the 45th day and term, the plasma concentration varied between 64 and 122 μg P/ml with an average of 89. Considerable differences could be found between litter mates. No correlation with gestation age was observed.

The average foetal concentration is thus twice as high as the maternal concentration. A certain correlation between the foetal and maternal concentrations was found, but on account of the limited material it was not statistically significant. It is evident that a passage of inorganic phosphate from mother to foetus must take place against the concentration gradient. About the electrochemical conditions nothing is known.

Foetal Phosphorus Retention.

The total phosphorus content was determined in the majority of the foetuses of the 18 animals in the present series and some additional foetuses from other experiments. The foetal age ranged from 29 days to term. The average for each litter has been plotted on Fig. 1. From the resulting curve the daily phosphorus retention has been calculated. It is shown on Fig. 2, where it is indicated by the curve. It is seen that the daily retention increases linearly from the 38th day of gestation, the daily increase being 1.06 mg.

Transfer of Phosphate to Foetus and Placenta.

The data necessary to calculate the amounts of inorganic phosphate transferred to the foetus and to the placenta are shown from two typical experiments in Table 1. The data for the other experiments will be reported in detail elsewhere (FUCHS 1957). It is seen that on the 43rd day of gestation about 3 mg is transferred to the foetus and 11 mg to the placenta per 24 hours when calculated from the results of an experiment of a duration of half an hour. At this stage of gestation the foetus retains about 8 mg phosphorus per 24 hours. On the 52nd day the foetal uptake is 13—15 mg, the placental uptake 16—19 mg, and the foetal retention about 17.5 mg per 24 hours.

The relation between the retained and transferred amounts is illustrated by Fig. 2, where the columns indicate the average transferred amounts for the litters from 18 experiments of duration from 15 to 50 minutes. The solid and hatched columns indicate the foetal uptake with labelled inorganic phosphate and with labelled donor plasma, respectively, and the open columns indicate the placental uptake. If the foetal uptake is regarded alone, it is seen that with one exception not enough is transferred to cover the foetal need for phosphorus. Only if the amounts taken up by the placentas are added to the foetal uptakes, do the

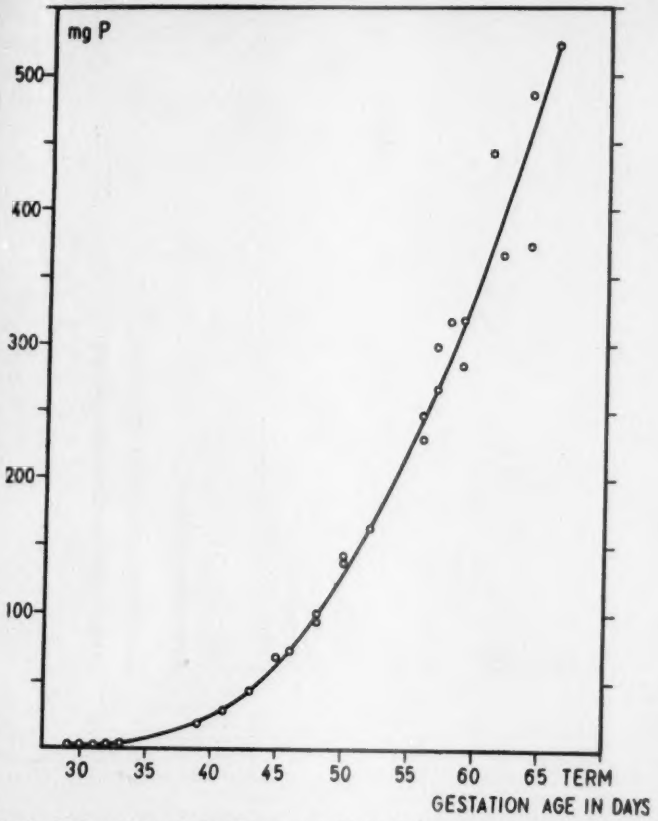

Fig. 1. The content of phosphorus in guinea pig foetuses at various stages of gestation. Each circle represents the average of a litter.

transferred amounts surpass the retained quantities of phosphorus which are indicated by the curve.

Discussion.

The experiments with radioactive phosphorus injected as inorganic phosphate and those with labelled donor plasma have not been carried out on the same days of gestation, but it is evident from Fig. 2 that they are in good agreement. Within the limits of the individual variations there is no appreciable

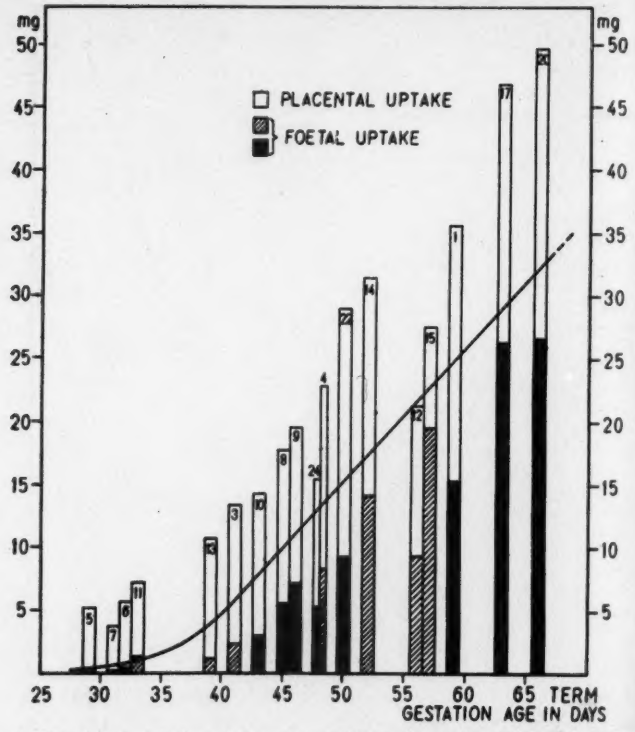

Fig. 2. The foetal and placental uptake of phosphate per day. The solid columns represent experiments with ^{32}P injected as inorganic phosphate, the hatched columns experiments with labelled donor plasma. The curve indicates the daily phosphorus retention in the foetuses. The numbers at the top of the columns indicate the numbers of the experiments. Exps. No. 4 and 24 were carried out on the same day of gestation, and the columns are made narrower to show them both on the diagram.

difference. It would therefore seem justified to conclude that if a labile, non-diffusible phosphorus compound, which chemically is determined as inorganic phosphate, does exist in the maternal plasma, it is either not present in such quantities as to invalidate the results obtained with the present methods, or it can be utilized for transfer to the foetus to the same extent as the inorganic plasma phosphate.

WILDE, COWIE and FLEXNER (1946) found for phosphate a safety factor — defined as the ratio between the transferred and retained amounts (FLEXNER and POHL 1941) — of unity

Table 1.

Data from two typical experiments. In Exp. No. 10 ^{32}P was injected as inorganic phosphate, while in Exp. No. 14 donor plasma labelled with ^{32}P was injected into the mother.

Experiment No.	10			14		
Gestation age	43 days			52 days		
Weight	780 g			1,077 g		
Foetus No.	1	2	3	1	2	3
Foetal weight (g)	14.36	13.91	14.54	40.9	39.1	42.4
Foetal P (mg)	41.5	42.0	45.3	169.4	158.7	
Placental wt.	4.99	4.79	5.82	5.61	5.55	5.82
Placental P	10.31	9.34	11.50	10.57	11.05	11.73
Maternal plasma P (μg/ml)		44.5			36.7	
Time of delivery (min.)	33	34.5	36	31	33	
Maternal plasma act. ($^{32}P_m$)	322	314	307	228	220	
Total act. in foetus ($^{32}P_{pf}$)	480	535	525	2,060	1,800	
Foetal uptake mg P/24 hrs. (P_f)	*2.92*	*3.17*	*3.05*	*15.40*	*12.88*	
Total act. in placenta	1,800	2,017	2,126	2,418	2,200	2,750
Act. in maternal blood in plac.	66	64	78	66	67	68
Act. in placental tissue ($^{32}P_{pl}$)	1,734	1.953	2,048	2,352	2,133	2,682
Placental uptake mg P/24 hrs. (P_{pl})	*10.46*	*11.47*	*11.89*	*17.44*	*15.50*	*19.14*

or less. The present results are in good agreement with this, the safety factor for the foetal uptake alone varying between 0.39 and 0.98 with one exception, in which it is 1.54. But when the placental uptake is added, the safety factor is definitely above unity, being 10 in the middle of gestation and decreasing gradually to about 1.5 at term. This means that enough phosphorus is supplied as inorganic phosphate to cover the foetal needs. Calculation of the safety factor facilitates comparison of experiments not carried out on the same day of gestation, especially during the last two weeks where the factor is rather constant.

In the half hour following the injection, the duration of most of the experiments, the placenta takes up very considerable amounts of radioactive phosphate from the maternal plasma, in the middle of gestation several times as much as the foetus, and near term about the same amount as the foetus in spite of the fact that the foetus may weigh fifteen times as much as the placenta at this stage. However, it is not permissible to consider

all the ^{32}P found in the placenta at the end of the experiment as taken up by the placental tissue. Up to 24 % of the placental weight is maternal blood (FUCHS 1953), and this cannot be removed by perfusion or washing without removing phosphate from the tissue. It is necessary to calculate the amount of ^{32}P in the maternal blood in the placenta from the previously determined blood volumes and the activity in the maternal blood. It only amounts to a few per cent of the total placental activity, and it has been deducted before the placental uptake of inorganic phosphate per day has been calculated. A correction for the ^{32}P in the foetal blood of the placenta has not been made; it would increase the foetal values slightly at the cost of the placenta, but the total height of the columns on Fig. 2 would not be changed.

Some of the labelled phosphate which is taken up by the placental tissue can be regarded as having displaced a similar amount of inactive phosphate to the foetal side, and it is this amount which must be added to the foetal uptake to give the total amount which has been transferred to the foetus. But some of the labelled phosphate in the placental tissue is taken up for the purpose of placental growth, and some of it probably enters in an exchange between the maternal plasma and the placental cells associated with placental metabolism, but without direct relation to the supply of phosphate to the foetus. Since the placental growth rate is slow as compared with the foetal, the amount retained in placental growth is small in comparison with the foetal retention, on the average 0.25 mg P per day. The phosphorus content per gram placenta is remarkably constant during the period studied, with an average of 2.0 mg P per gram wet weight. The amounts of inorganic phosphate and various organic phosphorus compounds in the placenta and their uptake of ^{32}P will be considered in another paper (FUCHS and FUCHS 1957 b).

Although it cannot be determined exactly how much of the placental uptake represents phosphate "in transfer" to the foetus, it can be concluded that the foetus receives enough inorganic phosphate through the placenta to cover what it needs for its development, but the safety margin is small, especially late in gestation. The experiments do not exclude the possibility that phosphorus is supplied to the foetus in other forms, for instance as phospholipids. NIELSON (1942) found that transfer of phospholipids, if any, must be very slow, but POPJÁK and BEECKMANS (1950) demonstrated that the maternal plasma phospholipids

do provide a source of phosphorus to the foetus, although they are not transferred as lipids, but are broken down in the placenta.

The phosphorus not needed in foetal growth must return to the mother in some form. The transfer of phosphate from foetus to mother has been studied in another series of experiments which will be reported separately (FUCHS and FUCHS 1957 a).

Summary.

In a study of the placental transfer of phosphorus in the guinea pig the transfer of inorganic phosphate from mother to foetus has been investigated from the 29th day to term with the aid of radioactive phosphorus. The inorganic phosphate in the maternal plasma was found to be the main source of phosphorus to the foetus. If most of the placental uptake of phosphate not needed in placental growth was considered to be transferred to the conceptus, enough inorganic phosphate is supplied to cover the foetal needs for phosphorus, but the safety margin is small, especially late in gestation.

The amount of phosphorus retained in foetal growth per day increases with advancing gestation, and the increase is linear from the 38th day to term. The amount retained in placental growth is nearly constant during the period studied.

If the maternal plasma, as our previous studies indicate, contains a labile, non-diffusible phosphorus compound, which is broken down to inorganic phosphate in the course of the chemical determination, it is either present in too small quantities to influence the results, or it is utilized by the conceptuses to the same extent as the inorganic phosphate in the maternal plasma.

The foetal plasma was found to contain twice as much inorganic phosphate as the maternal plasma. The transfer of phosphate from mother to foetus thus takes place against a concentration gradient.

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Studies on the Placental Transfer of Phosphate in the Guinea Pig.

II. The Transfer from Foetus to Mother.

By

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While the passage of substances from mother to foetus has been the subject of many investigations, less has been done to study the transfer in the opposite direction. This, of course, is due partly to technical difficulties, partly to the fact that it has been inferred that substances which are able to pass in one direction must also be able to move the opposite way if the concentration gradients are reversed.

A century ago, SAVORY (1858) was the first to prove the passage of a substance in the direction from foetus to mother in different animals by experiments with strychnine. This substance was injected into foetuses with intact placental circulation, and after a while an unmistakable toxic effect upon the mother animal could be observed. The mother died while the non-injected foetuses remained alive.

In a previous communication the transfer of inorganic phosphate from mother to foetus in the guinea pig has been reported (FUCHS and FUCHS 1957). In the present paper experiments are reported, designed to study whether there is any passage of inorganic phosphate from foetus to mother. The experiments were carried out with the aid of radioactive phosphorus which was injected in the form of inorganic phosphate into the umbilical vein of guinea pig foetuses with intact placental circulation.

Procedure.

Pregnant guinea pigs in the last stage of gestation were used. For anaesthesia Nembutal was injected intraperitoneally. The abdomen was opened by a midline incision, and one of the foetuses was delivered through a transverse incision in the uterus without interruption of the umbilical circulation. With a fine needle a small amount of highly active ^{32}P as inorganic phosphate was injected into the umbilical vein towards the foetus. A little bleeding was unavoidable after the withdrawal of the needle, but it usually stopped upon careful application of Gel-Foam without compression of the umbilical vessels. For a certain period of time (17—37 minutes) the foetus was then held in the hand in such a position that the pulsation of the umbilical arteries and the colour difference between arteries and vein in the cord could be watched. The foetus was kept warm and moist with warm saline, and no gasps or other signs of foetal distress were observed. At the end of the experimental period a blood sample was withdrawn from the umbilical vein, the cord was clamped and severed, and the foetus and the corresponding placenta with its subplacenta were removed. Hereupon the other foetuses and placentas were delivered, and finally a blood sample was withdrawn from the heart of the mother animal. The injected foetus as well as the litter mates were all alive at delivery and started gasping very quickly.

The foetuses and placentas were dried and ashed as previously described (Fuchs and Fuchs 1957), and phosphorus determinations and radiochemical measurements were likewise carried out as in the previous series.

Results.

For the calculation of the amount of inorganic phosphate transmitted from foetus to mother (P_m) the following equation (1) was used:

$$\frac{^{32}P_f}{P_f} = \frac{^{32}P_m}{P_m} = \frac{^{32}P_{pl}}{P_{pl}} \qquad (1)$$

P_f is the concentration of inorganic phosphate in the foetal plasma which is determined directly. $^{32}P_f$ denotes the average foetal plasma activity. Withdrawal of more than one blood sample from the fragile, easily collapsible umbilical vein is difficult, and consequently it has not been possible to establish a disappearance curve for ^{32}P in the foetal plasma. It had to be assumed that the foetal disappearance curve is identical with the maternal, and the few observations made support this assumption. Accordingly, the curve established for adult guinea pigs (Fuchs and Fuchs 1954) was used. The initial activity in

Table 1.

Results of four experiments on the passage of phosphate from foetus to mother.

Experiment No.	507	506	508	509
Weight of animal (g)	700	772	1,421	1,000
Gestation age (days)	55	60	61	65
No. of foetuses	2	2	5	2
Weight of injected foetus (g).	50.1	72.7	71.5	90
Weight of placenta (g)	4.58	6.55	6.3	11.2
Duration of experiment (min.)	17	37	30	19
Average act in foetal plasma ($^{32}P_f$)	38,600	12,600	10,000	30,000
Inorg. phosphate in foetal plasma (P_f) (μg P/ml)	90	103	95	67
Act. in non-injected foetuses		338		610
Act. in non-injected placentas.		668	166	1,390
Average act. in maternal plasma	60	56	13	75
Difference between injected and remaining act. ($^{32}P_m$)	19,300	14,810	5,300	18,000
Inorg. phosphate transferred from foetus to mother (P_m) (mg P/24 hours)	*3.24*	*4.67*	*2.42*	*3.04*
Act. in placenta of inj. foetus	29,900	23,100	12,400	72,000
Act. in foetal blood in placenta	5,610	7,070	4,280	13,350
Inorg. phosphate taken up by placenta (P_{pl}) (mg P/24 h.)	*4.80*	*5.07*	*3.69*	*9.90*

the foetal plasma was determined as the injected amount of ^{32}P divided by a plasma volume of 5 ml per 100 g foetal weight (FUCHS 1953). This includes the plasma in the cord and the foetal vessels of the placenta. The amount of ^{32}P transferred to the mother ($^{32}P_m$) was taken as the difference between the total injected activity and the activity remaining in the foetus and placenta at the end of the experiment. Since this difference only amounts to 1—3 % of the injected activity and the possible errors are at least of a similar order, the value arrived at can only be an approximation. Fortunately, it was possible to determine the $^{32}P_m$ in two other ways, partly through determinations of the activity in the maternal plasma, from which the average maternal plasma activity during the experiment could be estimated, and partly from the uptake of ^{32}P in the non-injected foetuses and their placentas. The quantity of ^{32}P necessary to obtain a certain average maternal plasma activity and to give a certain transfer to placentas and foetuses, when given as a single intravenous injection at a given gestation age and in an animal of a given

Table 2.

The amounts of phosphate transferred from foetus to mother and to placenta compared with the amounts of phosphate retained in foetal growth on the corresponding days. The last figures show the returned amounts in per cent of the retained plus the returned amounts.

Experiment No.	507	506	508	509
Gestation age (days)	55	60	61	65
Transferred from foetus to mother (mg P/day) (A)	3.24	4.67	2.42	3.04
Transferred from foetus to placenta (B)	4.80	5.07	3.69	9.90
Retained in foetal growth (C)	20.8	26.1	27.2	31.4
$\frac{A+B}{A+B+C} \times 100$	28	27	18	29

weight, was known from previous studies (FUCHS and FUCHS 1957). Although the ^{32}P is supplied continuously from the injected foetus in the present instance, the quantity is assumed to be of the same order. While definitely indicating a transfer of phosphate from foetus to mother, the first experiments were unsuccessful insofar as they did not allow any calculation of the transferred amounts. In three of the successful experiments, data were available for two of the three methods of calculation, and in a fourth experiment all three methods could be applied. The possibilities of errors are great with all the methods, but the results are in reasonable agreement. The data are shown in Table 1.

The placental uptake of inorganic phosphate from the foetal side (P_{pl}) is calculated from the total content of ^{32}P in the placenta ($^{32}P_{pl}$) and the $^{32}P_f$ and P_f values mentioned above. However, the content of ^{32}P in the blood of the foetal vessels of the placenta must be deducted from $^{32}P_{pl}$, since it has not left the foetal circulation. It is calculated on the basis of the activity in the foetal blood at the end of the experiment and a blood volume of the foetal vessels of the placenta of 0.2 ml per gram wet weight (FUCHS 1953). The amount of ^{32}P in the maternal blood of the placenta ought also to be subtracted and added to the maternal uptake, but since it is the combined maternal and placental uptake which is of interest, this correction is less important and has been disregarded.

In Table 2, the amounts of phosphate transferred per 24 hours to the mother and to the placenta from the foetal side are com-

pared with the amounts retained in foetal growth on the same days of gestation (FUCHS and FUCHS 1957). It is seen that the amounts returned from the foetus constitute on the average 25 % of the retained and returned amounts together.

Discussion.

Unfortunately, no experiments on the transfer of phosphate from mother to foetus were carried out on the same days of gestation as the four experiments on the transfer in the opposite direction described above. However, the results of the previous experiments (FUCHS and FUCHS 1957) can be compared with the present ones by calculating the ratios between the transferred amounts and the amounts retained in foetal growth on the corresponding days. With regard to the transfer from mother to foetus this ratio is designated the safety factor. In Fig. 1 the ratios for the present experiments are shown together with the safety factors for five previous experiments in the last part of gestation. The retained amounts are indicated as the horizontal line through 1.0, and when the amounts transferred from foetus to mother are added to the retained amounts, the sums become about equal to the amounts transferred from mother to foetus. Since it is not known how much of the placental uptake represents phosphate "in transfer", it is not possible to say, how close is the agreement. There might be a margin for transfer of other phosphorus compounds, *e. g.*, phospholipids, but such a transfer does not appear to play a major rôle in the supply of phosphorus to the foetus.

As previously described (FUCHS and FUCHS 1957), the inorganic phosphate concentration is about twice as high in the foetal as in the maternal plasma. Transfer of phosphate from foetus to mother thus takes place along the concentration gradient and consequently could be effectuated by diffusion, provided this is not prevented by an electrochemical gradient in the opposite direction.

The rates of transfer of inorganic phosphate across the placental barrier in both directions will be discussed elsewhere (FUCHS 1957).

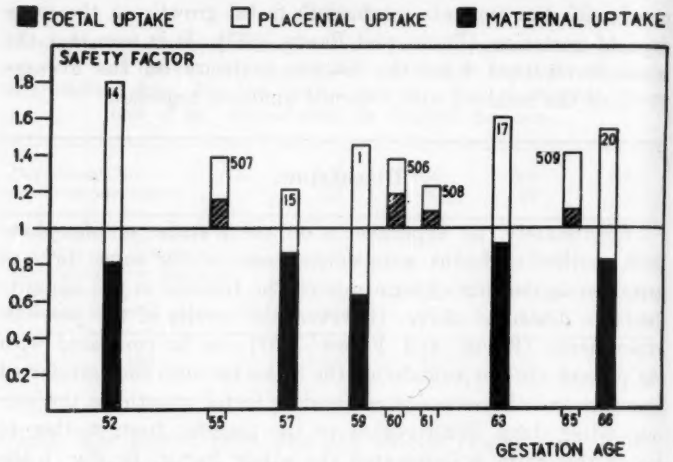

Fig. 1. Experiments on transfer from foetus to mother compared with previous experiments on transfer from mother to foetus by comparison of the ratios between transferred amounts and amounts retained in foetal growth. For transfer from mother to foetus this ratio is called the safety factor. The retained amounts have been plotted = 1, and the amounts transferred from foetus to mother have been added to the retained amounts. Together they are about equal to the quantities transferred from mother to foetus. The numbers at the top of the columns indicate the numbers of the experiments.

Summary.

In a study of the placental transfer of phosphorus in the guinea pig the transfer of inorganic phosphate from foetus to mother has been investigated in the last part of gestation with the aid of radioactive phosphorus. The quantity transferred in this direction across the placenta is approximately 25 % of the amount which is transmitted from mother to foetus at the same stage of gestation. The amount transferred from mother to foetus is about equal to the amount retained in foetal growth plus the amount returned to the mother. The return of phosphate from foetus to mother is facilitated by the concentration gradient from the foetal to the maternal plasma.

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The Effect of Vagotomy on Gastric Motor Responses to Drugs in Dogs.

By

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During the past few decades, extensive studies have been made of the supersensitivity to drugs exhibited by denervated plain muscle. On the whole, the results support the view that plain muscle, like striate muscle, autonomic ganglia and secretory cells, obeys the "Law of Denervation" (CANNON 1939). The plain muscle structures most thoroughly investigated from this point of view have been the nictitating membrane, the pupillary muscle and the small blood vessels; the available information was reviewed by CANNON and ROSENBLUETH (1949).

However, comparatively little attention has been paid to the effect of denervation on the sensitivity to drugs of the plain muscle in the alimentary tract, although in fact most of the plain muscle in the body is located there. One reason for this lies in the difficulty of making satisfactory comparisons between normal and denervated preparations, because of the absence of paired organs. Another problem arises in the considerable and variable basal activity observed in the various regions of the alimentary tract, particularly in conscious animals.

There are nevertheless a few reports concerning the effect of adrenaline on intestinal motility in relation to denervation. An increased inhibitory effect of adrenaline on the isolated rabbit intestine after denervation was demonstrated by LUCO (1937) and by DRAKE, RENSHAW and THIÈNES (1939). Similar results

were obtained by YOUMANS, KARSTENS and AUMANN (1942) who studied dogs with different kinds of intestinal fistulae and various combinations of denervation. In most cases denervation was performed at the root of the mesentery, which would constitute a preganglionic parasympathetic and a postganglionic sympathetic denervation. In a few experiments vagotomy alone was performed, and in these cases little or no increase in the inhibitory effect of adrenaline on intestinal motility was demonstrable. LUCO (1937) determined the sensitivity of intestinal muscle to acetylcholine after denervation in a few experiments, and stated that no supersensitivity to this agent was observed.

Regarding sensitivity of gastric plain muscle to drugs, KURODA (1924) states that increased motor responses to adrenaline and acetylcholine are obtained after denervation. Apart from this report vhich is based on observations on isolated fundic strips from one dog only, previous investigations have given no evidence of increased sensitivity of gastric muscle to drugs after denervation. BORCHER (1921) in studies on cats using the abdominal window technique found that pilocarpine had the same effect after vagotomy as before; the doses used were not specified. WATANABE (1924) studied the effect of acetylcholine, pilocarpine and adrenaline on gastric motility in dogs, using radiographic methods. This author found that, in normal animals, acetylcholine increased tonus, while pilocarpine increased peristalsis; these actions were unaltered by vagotomy. Adrenaline, on the other hand, showed mixed excitatory and inhibitory effects on normal stomach, but produced inhibition only after vagotomy. In these experiments the doses of the drugs administered were large (several hundred times threshold) and a possible sensitization after vagotomy could therefore not be revealed. The "Law of Denervation" in relation to gastric function has been discussed by ANTIA, ROSIERE, ROBERTSON and GROSSMAN (1951) who determined the effects of vagotomy, especially upon gastric secretion. They observed a decrease in the secretory responses to histamine and urecholine during the first few months after vagotomy and regarded this as an apparent "violation" of the law. Exceptions to Cannon's law were discussed, and the fact that vagotomy is a preganglionic denervation was suggested as a possible reason for their failure to observe sensitization.

In studies on the sensitization of effector organs to chemical agents by denervation, there are certain considerations which

must be borne in mind when planning the experiments and interpreting the results. The maximal response to stimulation is not increased by denervation, but sensitization can be detected as a decrease in threshold of the drug or as an increase in response to submaximal doses. It follows that the doses used for sensitization to become apparent should be between threshold and the smallest dose giving a maximal response in the normally innervated structure. Further, sensitivity to drugs and also the degree of supersensitivity observed after denervation varies between individual animals, so that in order to avoid using large series it is highly desirable to employ a technique which enables repeated observations to be made on the same animal.

In the experiments to be described, these special conditions have been taken into consideration. A study has been made of the sensitivity of the gastric muscle to drugs before and after vagotomy in dogs, the period of observations extending over 60 days before nerve section and up to 200 days afterwards. In most experiments, the basal motor activity of the stomach was abolished by temporary anaesthesia secured by intravenous nembutal; some experiments of similar type were performed on conscious animals.

Methods.

A total of 238 experiments on 18 dogs weighing 10—32 kg were performed. The main series of observations, which are presented in detailed form includes 145 experiments performed on 11 dogs. Repeated observations were made of the effects of adrenaline, mecholyl (metacholine chloride)[1] and carbachol (carbaminoylcholine chloride) on gastric motility in the anaesthetized animal, before and after vagotomy. Similar observations were made on 4 dogs subjected to a "sham" operation (31 experiments). Two of these dogs were used some months afterwards for vagotomy experiments. A further 5 dogs were studied before and after vagotomy in the same way as those above, except that the experiments were performed on the conscious animal and the only drugs studied were pilocarpine (23 experiments on 2 dogs) and carbachol (39 experiments on 3 dogs).

Operative preparation. A wide-bore silver cannula, closed by a screw-cap, was installed through an upper midline incision in the lower part of the gastric corpus, near the greater curvature. The animals were ready for experiments 2—3 weeks after this operation.

Vagotomy was performed by the transthoracic route under nembutal

[1] Thanks are due to Erik Lindblom and Co, Stockholm for generous supply of the drug (Mecholyl-chlorid, Sharp & Dohme).

anaesthesia (30 mg/kg intravenously) using artificial respiration, the lower part of the oesophagus being exposed by resection of the 6th rib on the left side. All visible vagal branches (2—4) were divided between ligatures with resection of 2—3 cm. In addition, the superficial layer of the muscular wall was removed from the circumference of the oesophagus in order to interrupt any buried nerve fibres.

The "sham" operation was performed in exactly the same way, even to dissection of the main trunks from the surface of the oesophagus as though in preparation for section. The nerves were, however, not divided and the wall of the oesophagus was not incised.

All animals were given penicillin for 4—6 days after operation.

Gastric motility studies. Comparisons of gastric motility during these experiments were performed by the following strictly standardized method. The animal was fasted 24 hours before hand. Four to eight hours before the experiment, which was usually done in the afternoon, the stomach was washed out with warm water and the cannula left open. For the experiment, the dog was placed in a Pavlov stand and a latex rubber balloon connected to a bromoform manometer introduced into the stomach. The balloon was then inflated with 100 ml of air, an amount which outside of the body just filled the balloon without stretching it. Thus the elasticity of the balloon itself was not concerned in the pressures recorded. Inflation of the balloon caused a fairly constant rise in manometer pressure with more or less regular fluctuations which were recorded on the kymograph for some minutes before any injections were made. Injections were given into a long polythene tube connected to a cannula in a limb vein (usually the small saphenous vein), thus avoiding to disturb the animal during the experiment.

In the main series of experiments the animal was anaesthetized with nembutal (30 mg/kg intravenously). While under anaesthesia the dogs were maintained in a standing or hanging position, supported by straps under the upper part of the thorax and the lower part of the abdomen. The trachea was intubated to maintain a clear airway.

Saline solution of adrenaline, mecholyl and carbachol were injected in increasing doses from subthreshold up to 1 μg/kg for carbachol and up to 5 μg/kg for adrenaline and mecholyl. The concentrations of the solutions employed were 1 μg/ml and 10 μg/ml. The volumes injected varied between 1 and 16 ml of fluid according to dosage and weight of the animal. Four ml of saline was always given after each injection (the volume of the polythene tube being 2 ml). Control injections of only saline solution had no effect.

In the series of experiments done with the animals in the waking state the procedure was the same except that nembutal was not injected and only pilocarpine and carbachol were studied.

Results.

The results to be described are in the main those derived from the 11 dogs subjected to repeated experiments under anaesthesia

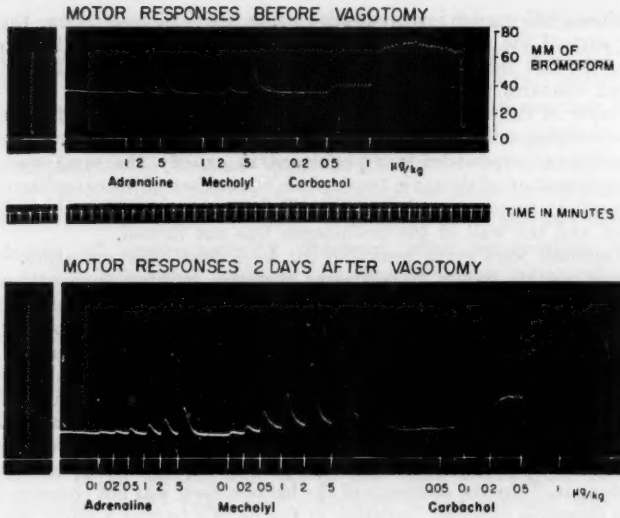

Fig. 1. Records from two separate experiments on one dog, with motor responses to different doses of the drugs under nembutal anaesthesia. The appearance of the tracing before anaesthesia was given is presented in the separate sections at the beginning.

before and after vagotomy, with observation of the responses to small doses of adrenaline, mecholyl and carbachol. The sensitivity to the drugs used has been expressed in two ways:

1. The threshold dose for motor responses, *i. e.*, the smallest doses producing a definite increase in the pressure recorded by the bromoform manometer.

2. The size of the response (mm of bromoform) to a given dose. The values for the size of the response to each dose were obtained by measuring the maximal increase in manometer pressure from the baseline level.

Beside lowered threshold, and increased size of the response to the different doses, increase in duration of the response was usually observed after vagotomy. The values for duration have, however, not been made use of when estimating the increase in sensitivity.

A typical change in responsiveness following vagotomy is presented in fig. 1, which gives the records from two experiments on the same animal. After induction of anaesthesia a constant

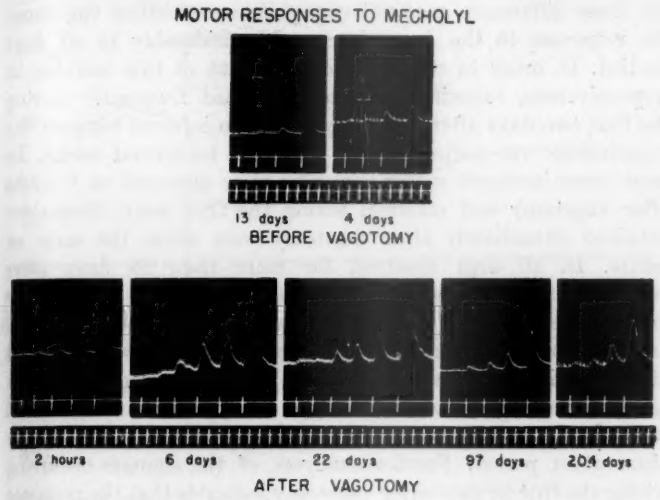

Fig. 2. Additional estimations of the responses to mecholyl on the same dog as in fig. 1. Increasing doses from subthreshold up to 5 μg/kg.

tone of the apparently inactive stomach is indicated by the smooth base-line showing minute deflections due to respiratory movements. In all experiments on anaesthetized dogs carbachol, mecholyl and adrenaline gave rise to positive responses *i. e.* increase in manometer pressure. When mecholyl or adrenaline was given, the pressure started to rise a few seconds after injection and had usually reached the maximal value within 30 seconds, whereafter it returned to the basal pressure within 1—2 minutes. For carbachol the increase was often more gradual and the pressure remained on a high level for several minutes and even up to more than one hour. In addition to the increase in pressure, the bigger doses of carbachol provoked regular fluctuations in pressure, indicating peristaltic movements in the stomach.

In order to illustrate the type of responsiveness occurring in these experiments, a series of tests with mecholyl before and after vagotomy are presented in fig. 2. The curves shown in figures 1 and 2 were obtained from one and the same dog. During the preoperative period the responses obtained from repeated tests were fairly uniform in one and the same animal. Between different dogs variations occurred in the preoperative responsiveness,

but these differences were not very pronounced. After vagotomy the responses to the drugs increased considerably in all dogs studied. In order to study the early course of this increase in responsiveness, experiments were performed frequently during the first two days after vagotomy; later the interval between the experiments was varied from a few days to several weeks. In most cases increased motor responses were apparent at 6 hours after vagotomy and maximal within the first week. Responses obtained immediately after vagotomy were about the same as before. In all dogs observed for more than 20 days after vagotomy, the responses were found to decrease during the following period. In most cases, and especially for carbachol and mecholyl a rapid decline in responsiveness was observed at some time between 20 and 60 days after vagotomy. After this fall, the values remained fairly constant at a level somewhat above the preoperative values until the end of the observation period. Further analyses of the changes occurring within the first 20 days after vagotomy indicates that the response after the initial increase is diminished and then increases again; however, this feature could be observed clearly in five dogs only. For adrenaline the responses were more regular, with an early increase to values which in many cases were maintained during the whole period.

Atropine was given in some experiments (1 mg/kg intravenously) and was found to abolish the responses to carbachol and mecholyl; the responses to adrenaline remained unchanged. Dihydroergotamine (0.5 mg/kg intravenously), on the other hand, reduced or abolished the responses to adrenaline. Hexamethonium (5 mg/kg intravenously) had no effect on the responses to mecholyl and adrenaline, but reduced the response to carbachol during 1—2 hours without abolishing it completely.

In order to illustrate the general changes observed in these studies, the total number of observations made on threshold and motor responses to different doses of carbachol, mecholyl and adrenaline are presented as mean curves in figures 3—6. Mean values and standard error of the mean are calculated from the number of observations within each time interval. The details of the early phase (up to 20 days), as revealed in the five dogs mentioned above, are not shown by these curves; but the pronounced regression between 20 and 60 days after vagotomy which was observed in nearly all cases is well illustrated by the

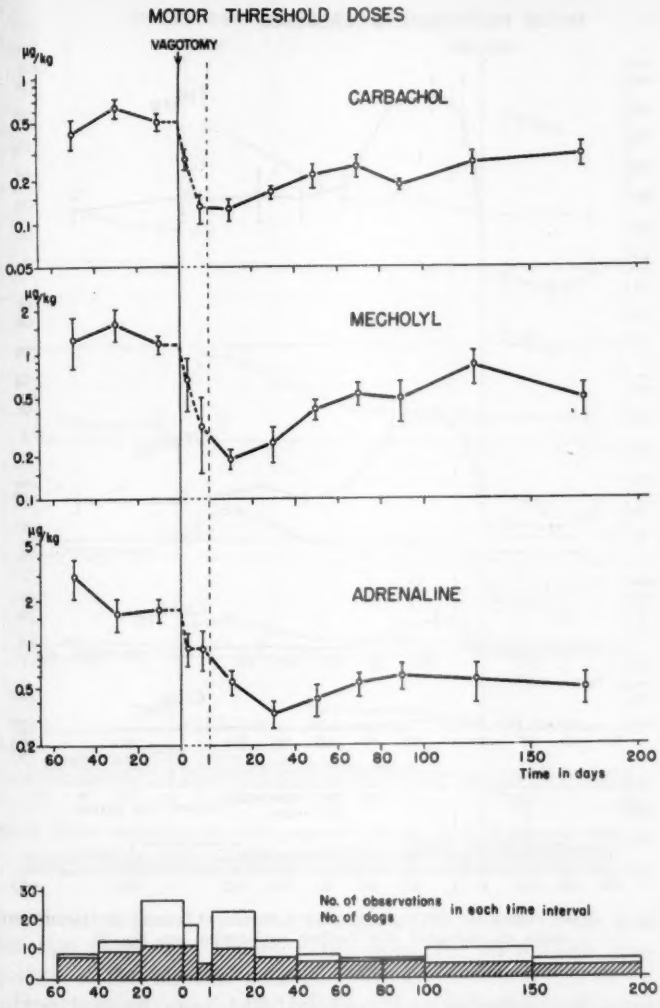

Fig. 3. Mean curves for motor threshold before and after vagotomy. Vertical bars through the points give the standard error of the mean calculated from all observations within each time interval. The columns at the bottom give the number of experiments (whole column) and number of dogs (shaded column) in each time interval. Time scale is enlarged for the first postoperative day.

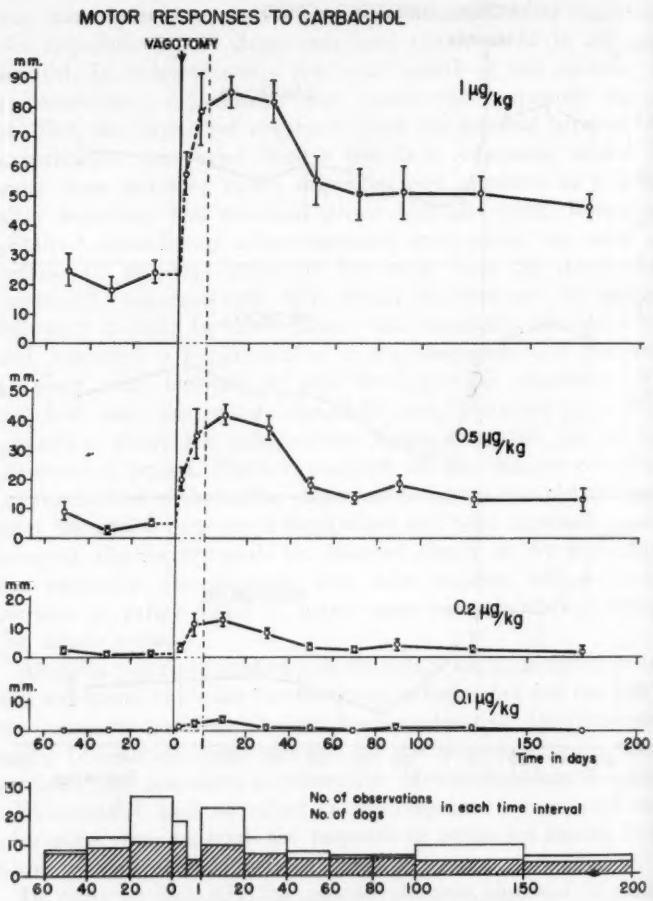

Fig. 4. Mean curves for the motor responses, in mm of bromoform, to different doses of carbachol (for further explanation see fig. 3).

curves for carbachol and mecholyl. The more gradual return observed with adrenaline is also apparent in the mean curves both for threshold and motor responses to this substance. The decrease in number of dogs during the postoperative period is due to the difficulty to keep the animals alive during the repeated experiments. No animal was deliberately sacrificed during the

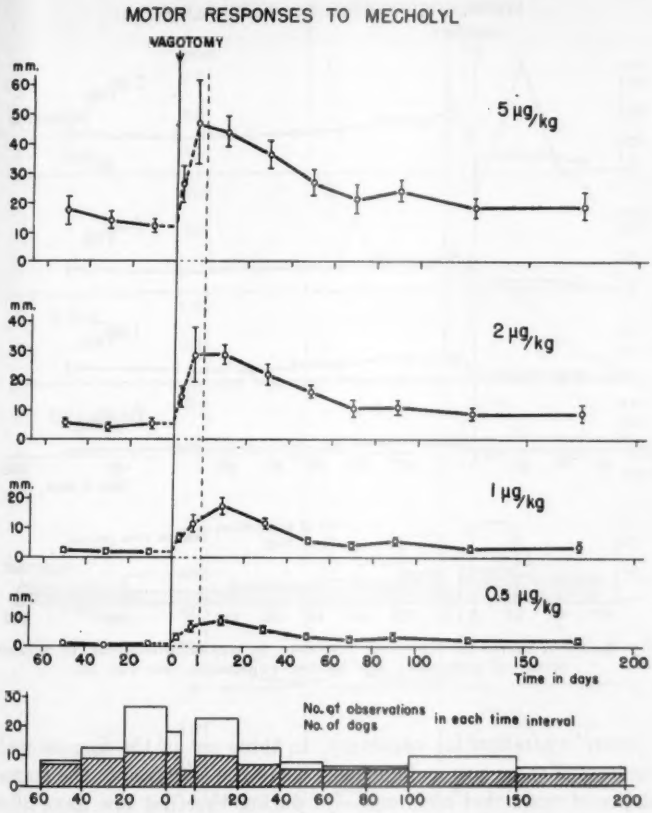

Fig. 5. Mean curves for the motor responses, in mm of bromoform, to different doses of mecholyl (for further explanation see fig. 3).

postoperative period of up to 200 days. The course of the sensitization curve was generally the same for all animals irrespective of the survival period.

The sensitivity to l-noradrenaline was estimated before and after vagotomy in one of the dogs included in the series. The responses showed an increase of approximately the same degree as was found for adrenaline.

Effects of the "sham" operation. Repeated observations similar to those reported above were made in the dogs subjected to the

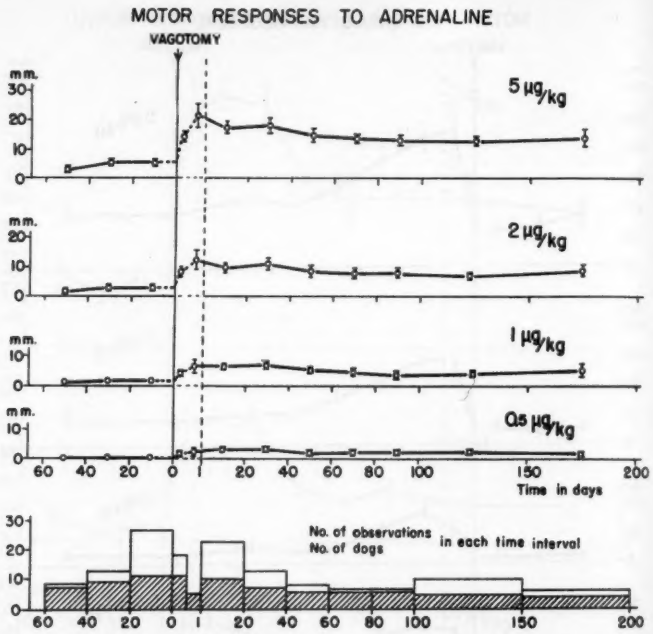

Fig. 6. Mean curves for the motor responses, in mm of bromoform, to different doses of adrenaline (for further explanation see fig. 3).

"sham" operation for vagotomy. In three out of the four animals so treated there was a slight increase in the response to the largest doses of carbachol and mecholyl during the first few days after operation. No increase to adrenaline was observed in any experiment. Fig. 7 shows the results obtained with one animal of this group which was vagotomised 63 days after the "sham" operation. This was the dog in which the biggest effect of "sham" operation was observed. The curves, representing the responses to the three largest doses of carbachol, mecholyl and adrenaline, exhibit an obvious difference in the degree of increase and in time-course after the two operations.

Experiments on conscious dogs. The series of experiments in which the sensitivity to pilocarpine and carbachol was determined on conscious animals before and after vagotomy actually constituted the first part of the investigation. However, owing

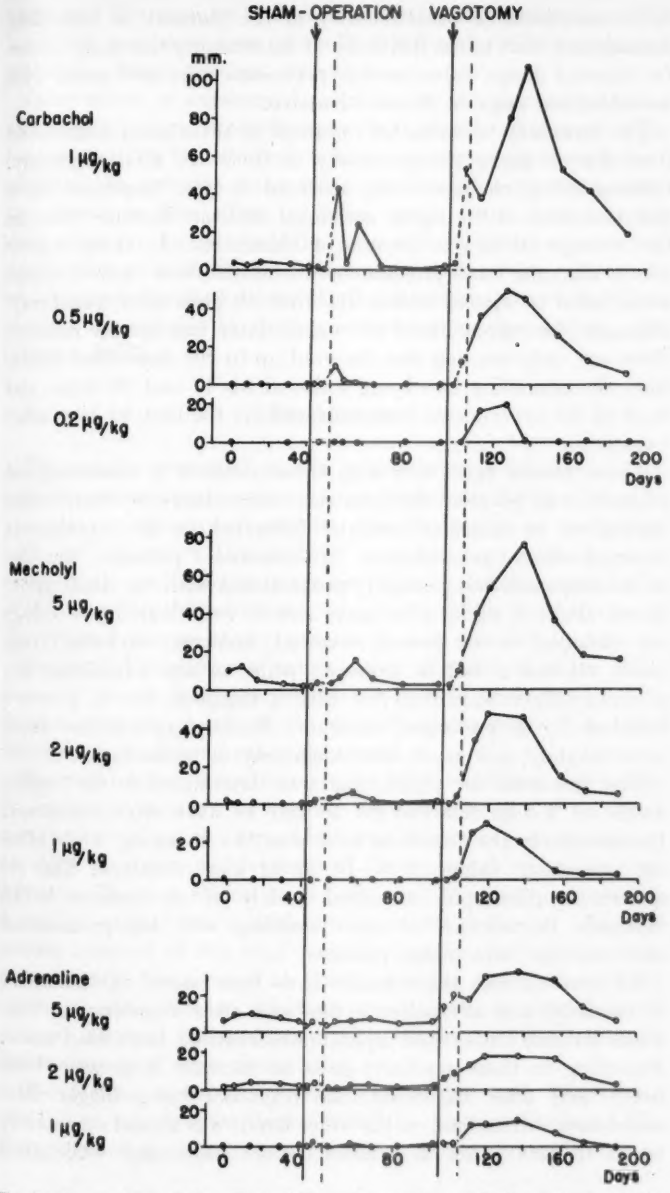

Fig. 7. Comparison of the effect of "sham" operation and of vagotomy on the motor responses to drugs. Experiments on one dog. (End of first postoperative days indicated by the dotted lines.)

to the considerable basal activity of the stomach in conscious animals and thus to the difficulty of determining threshold values for injected drugs, subsequent experiments were performed with anaesthetized dogs, as described above.

The threshold to carbachol obtained in these cases diminished from 0.2—1 μg/kg preoperatively to 0.05—0.2 after vagotomy. Corresponding changes were observed in the responses to a standard dose of 0.5 μg/kg carbachol with an increase from on the average 20 mm to 50 mm of bromoform. In these experiments also, the most pronounced deviations from control values were found to appear within the first 20 days after vagotomy, although the regression of the values later was not so marked. However, only one dog was observed up to 200 days after operation, the remaining two being followed for 17 and 90 days, and most of the experiments were made within the first 40 days after vagotomy.

These results agree well with those obtained in anaesthetized animals, but peculiar irregularities were observed when drugs were given to conscious animals. Carbachol, in the experiments reported above, gave increase in manometer pressure, the size of the responses being roughly proportional with the doses given (doses above 1 μg/kg often gave rise to vomiting). In two dogs not included in the present material, however, carbachol regularly elicited a fall in pressure, while in one additional dog a mixed effect was observed with a transient rise in pressure followed by a prolonged decrease. Similar experiments were, unfortunately, not made after vagotomy in these dogs.

The threshold for pilocarpine was determined in 23 experiments on 2 dogs followed for 58 and 89 days after vagotomy. Preoperatively the threshold level was 20—50 μg/kg, while after the operation values of 5—10 μg/kg were obtained. The responses to pilocarpine consisted mainly of an increase in the rhythmic fluctuations on the recording with less pronounced effect on the manometer pressure.

No systematical observations have been made on the effect of mecholyl and adrenaline before and after vagotomy in conscious animal; but several separate observations have been made. According to these mecholyl gave an increase in pressure both before and after vagotomy, the responses being bigger after vagotomy. Adrenaline, on the other hand, was almost exclusively found to give a fall in pressure before vagotomy, while after

vagotomy the response was reversed to an augmentory one. The inhibitory responses were generally found to be most pronounced when the basal pressure was high.

Other effects of vagotomy. In addition to the augmented motor responses to drugs, vagotomy gave rise to occasional increase in the basal activity of the stomach, both in conscious and anaesthetized animals; the increase being most pronounced some weeks after the operation. The effect on basal, gastric tone, however, was very slight as far as could be judged from these experiments. Among the general effects of vagotomy, the most prominent one was frequent vomiting during the first postoperative weeks. These changes are subject to further considerations in another paper (MUREN 1957).

Discussion.

The results presented in this paper provide evidence of increased gastric motor responses to carbachol, mecholyl, adrenaline and pilocarpine after vagotomy. This finding is in accordance with the "Law of Denervation" in that preganglionic parasympathetic nerve section gives rise to an unspecific sensitization of the thus denervated effector organ. A possible sensitization of the isolated postganglionic neurone is not revealed by this investigation. The degree of sensitization observed is of the order generally found to appear in smooth muscle after denervation. In some respects, however, the present results differ from those obtained in studies on other structures.

The rapid onset of supersensitivity after vagotomy can hardly be explained in terms of ensuing degeneration of the sectioned nerves. According to current opinion, however, supersensitivity develops in structures deprived of their normal stimulating influences. There is reason to believe that the gastric muscle is normally subject to considerable nervous influence and the sudden removal of this may possibly explain the rapid increase in sensitivity. YOUMANS et al. (1942), working with the inhibitory action of adrenaline on intestinal smooth muscle also observed a rapid increase in sensitivity with maximal values obtained within two days after denervation.

The results reported by BORCHER (1921) and WATANABE (1924) are not in accordance with the present findings. The divergence of the results can be explained by the difference in

experimental methods employed. As mentioned in the introduction, supersensitivity after denervation will not become apparent unless special conditions, including dosage of drugs and methods of recording the effects, are regarded.

The decline in supersensitivity observed in the present experiments some weeks after vagotomy, could reasonably be attributed to a regeneration of vagal fibres. It is, however, doubtful whether regeneration can take place within such a short time interval. Another possible explanation is that activities aroused in the postganglionic neuron after vagotomy are able to suppress the supersensitivity of the preganglionically denervated effector cells. These problems are further discussed in another paper (MUREN 1957).

The slight increase in sensitivity observed during the first few days after "sham" operations may be attributed to a transient disturbance of conduction in the vagus nerves, consequent upon the manipulations involved in their dissection from the oesophagus. A contributing factor was perhaps the long-continued anaesthesia which was necessitated by the frequent experiments performed during the first two postoperative days. It is tempting to assume that a similar mechanism was responsible for the early, transient increase in responsiveness observed after vagotomy in some cases.

The data obtained from the experiments in which the sensitivity to carbachol was determined on conscious animals before and after vagotomy agree well with those obtained in the experiments of similar nature performed under anaesthesia, indicating that nembutal does not influence the sensitivity of gastric muscle to carbachol. These results, however, are complicated by the finding, in other dogs, of an inhibitory effect exerted by carbachol. With adrenaline an inhibitory effect was regularly obtained in conscious animals. The reversal of the adrenaline effect, brought about by barbiturates, has previously been discussed by MCSWINEY & BROWN (1926, 1932) and others, but no conclusive explanation to the problem has been arrived at. The results of the present investigation suggest that vagotomy also brings about a reversal of the adrenaline effect. These phenomena are presumably not directly related to the process of sensitization, but they obviously interfere with the estimation of gastric motor responses to drugs in conscious animals. Further work on this subject seems desirable.

The experiments on anaesthetized animals, indicate that the increase in motor responses to drugs observed after vagotomy are due to an increased sensitivity of the plain muscle cell. There is no evidence in favour of the assumption that changes in basal tone of the muscle are responsible for the increased effects. As the responses immediately after vagotomy were the same as before, the supersensitivity can hardly be due to the mere exclusion of possible inhibiting influence from the vagus. Vascular effects may be involved in the process, but information about this can not be obtained from the present experiments. By using isolated preparations it should be possible to exclude this factor. Further information about the significance of stimulating influence for the sensitivity of the effector organ may be obtained by treating the animals with pharmacologically blocking or stimulating agents.

Summary.

In gastric fistula dogs the gastric motor responses to carbachol, mecholyl and adrenaline were recorded under nembutal anaesthesia in repeated experiments before and after transthoracic vagotomy. Observations were made up to 200 days after operation.

The threshold doses of the drugs mentioned for motor responses were markedly lowered by vagotomy and the responses to graded doses showed a corresponding increase as compared with preoperative responses.

Increased sensitivity was usually apparent within the first 24 hours after vagotomy and maximal values were in most cases attained within the first 20 days. Later on, a decline in supersensitivity was observed, but preoperative values were not reached within 200 days after vagotomy. With carbachol and mecholyl the decline was pronounced; with adrenaline it was very slight.

Similar experiments were performed on conscious dogs, using carbachol and pilocarpine. The results for carbachol agreed closely with those obtained in experiments performed under nembutal. The threshold dose of pilocarpine was found to be considerably reduced after vagotomy.

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